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Influence of Muscle Length on the Mechanical Parameters of Myocardial Contraction

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Abstract

NILSSON E *Influence of muscle length on the mechanical parameters of myocardial contraction* Acta physiol scand 1972 *85* 1—23

Stiffness determinations were made on rabbit papillary muscles at different lengths Both in resting p eparations and in muscles contract ng at lengths where resting force was very low compared with active force the stiffness increased recti linearly with force At any force level the stiffness of a preparation was greater at rest than during activity —Force velocity curves were determined at different muscle lengths and when necessary data for the whole preparation were corrected for the influence of the parallel elasticity in order to derive the force velocity curves of the contractile element alone V_m and P_0 of the contractile element (*i.e* its shortening velocity at zero load and its maximum force producing capacity) determined at peak isometric force were increased approximately in parallel by elongation at short muscle lengths whereas at lengths near the optimum for active force V_{max} was influenced slightly more than P_0 by length changes —The time course of the active state was crit cally dependent upon muscle length an increase in l ngth prolonged the time from stimulat on to 60 decay of the active state without significantly altering the time course of its rising phase

It has been found that in single skeletal muscle fibres of the frog the shortening velocity at a low force decreases with decreasing sarcomere length below the length optimum for isometric force whereas at greater sarcomere lengths the shortening velocity remains approximately constant (Gordon Huxley and Julian 1966) On the same preparation it has also been shown that the force during an isometric tetanus is dependent in a characteristic way upon the amount of overlap between the two sets of interdigitating filaments in the myofibrils (Ramsey and Street 1940 Gordon Huxley and Julian 1966 Edman 1966) In studies on cardiac tissues aimed at relating isometric force and shortening velocity to sarcomere length one has to consider difficulties raised by the complex structure of isolated myocardial preparations Additional problems arise due to the facts that myocardial cells are not fully activated during an ordinary contraction cycle and that optimum active force is normally generated in myocardium at high resting force It has not been possible so far to analyse the force velocity relation in the myocardium as a function of sarcomere

length However, the dependence of the force velocity relation on the overall muscle length has been studied on papillary muscles of the cat and divergent results have been obtained In an early study (Sonnenblick 1962) it was concluded that P_0 (defined by Sonnenblick as the peak of the isometric active force plus the resting force) increased with increasing muscle length whereas V_{max} (the shortening velocity of the muscle at zero load derived by extrapolation from his experimental data) was unaffected by changes in length of the preparation These conclusions, however, were reached from velocity measurements during afterloaded contractions It should be noted that the various points on one force velocity curve obtained in this way refer to different degrees of activity of the contractile element and to different lengths of this element Contrary to these results Noble Bowen and Hefner (1969) demonstrated a marked length dependence of V_{max} in papillary muscles by means of a quick release technique With this technique, all measurements used for determination of one force velocity curve can be related to the same degree of activity of the contractile system However it is rather difficult to interpret the results reported by Noble Hefner and Bowen (1969) because of their finding that force velocity curves were non hyperbolic at lengths below the optimum for active force Therefore it was considered of interest to reinvestigate the length dependence of force velocity relation of isolated myocardial preparations with the aid of the quick release technique utilizing critical damping described previously from this laboratory (Edman and Nilsson 1968 1969) (This technique will be referred to as the damped release' method in subsequent sections) With this method all determinations for one curve are performed at the same phase of the contraction cycle and at nearly the same length of the contractile element Such force velocity curves have a hyperbolic shape (Edman and Nilsson 1968 1972) In the present study, force velocity measurements were made at the moment of the peak of the isometric twitch, and when necessary the curves obtained in this way were corrected for the influence of parallel elasticity to produce force velocity curves of the contractile element alone

It has been demonstrated in frog skeletal muscle (Edman and Kiessling 1971) that active movement causes a decrease in the intensity of the active state and results reported by Joyce Rack and Westbury (1969) seem to indicate that the same effect is produced in mammalian skeletal muscle Furthermore it has also been shown that the duration of the active state is less when contraction is initiated at shorter sarcomere lengths than at longer (Edman and Kiessling 1971) In a previous study on rabbit papillary muscle it was demonstrated that shortening of the contractile system during activity reduced both the intensity and the duration of the active state (Edman and Nilsson 1968) But it was not possible in these experiments to differentiate between the influence of muscle length and the effect of movement *per se* on the active state Recently it was shown that active movement depresses the intensity of the active state in myocardium without significantly altering its duration, whereas the duration of the active state is abbreviated when the resting length of the preparation is reduced (Edman and Nilsson 1971) In the present investigation the influence of initial muscle length upon the time course of the active state in rabbit papillary

muscles has been studied in further detail Evidence will be presented that the duration of the active state measured at 60 % of peak activity decreases with decreasing muscle length over a wide length region

Methods

Preparation and recording device Papillary muscles were dissected from the right ventricle of rabbits (weight 0 8–1.2 kg) which were heparinized immediately before killing The papillary muscles were approximately cylindrical except for a tapered portion (about 10–20) near the tendon The slack length of the preparations used in this study was 3 5–6 5 mm the greatest diameter of the preparations was 0 9 mm. The preparation was mounted vertically in a thermostated Perspex bath as previously described (Edman and Nilsson 1968 1969) The lower end of the preparation (the ventricular wall end) was fixed to a force transducer (RCA 5734) and the upper end was connected to an isotonic lever via a straightened steel wire The lever could be locked against an upper stop screw by means of a catch which could be withdrawn at any preset time during contraction to enable isotonic shortening after a preceding isometric phase The load of the preapration was set with a spring attached to the lever on the opposite side of the fulcrum The movements of the lever were damped by means of a dashpot which consisted of a peg extending down from the lever into a cup filled with silicon oil The degree of damping could be varied by using silicon oils of different viscosities and by varying the depth of immersion of the peg into the oil The mechanical characteristics of the recording device are given in Appendix I For details concerning stimulation of the preparation production of release and recording of the signals from the tension and the displacement transducer see Edman and Nilsson (1968)

Compliance of the recording system The compliance of the platinum loops and silk threads used for connecting the preparation to the recording device measured in the absence of a papillary muscle was 14 μ/10 dyn In the calculation of the natural frequency of the length recording system (Appendix I) the total compliance of the system i e the compliance of the isotonic lever (5 and 3 μ/10 dyn for lever I and lever II respectively) and of the attachements of the preparation was taken as 20 μ/10 dyn This figure probably represents an upper limit because microscopic measurements with the preparation mounted in the recording chamber indicated a slightly lower compliance of the attachements than stated above (In two experiments a mean value of 10 μ/10 dyn was observed.

Solutions A Ringer s solution of the following composition was used (mM) NaCl 170 KCl 4 NaH PO 1 5 CaCl 2 MgCl 1 5 glucose 3 3 The pH of the solution was kept at 7 4–7 5 by continuous aeration with a gas mixture containing 95 O +5 CO Glass-distilled water was used for washing of the glassware and for preparation of solutions All chemicals used were of analytical grade

The experiments were carried out at temperatures of 26–28 C in each experiment the temperature was kept constant with n±0 7 C The preparation was stimulated at a frequency of 30 beats per minute unless otherwise stated and each muscle was stimulated for at least one hour before the experiment was started

Calculation of constants a and b in Hill's equation The graphical method of Katz (1939) was used for fitting force velocity curves to experimental data Successive determinations of constants a and b usually differed less than ±5

Statistics Student s t test was used in statistical analysis When comparing slopes of 2 regression lines the variances around the 2 lines were not assumed to be equal.

Results

I *Stiffness force relations of the series elastic and the parallel elastic element*

Several attempts have been made to describe the mechanical properties of isolated myocardial preparations in terms of mechanical models Fig 1 shows two of these models denoted model I and model II In the present study it will be assumed that the contractile element is freely extensible at rest and consequently does not bear resting force Model I and II are frequently although somewhat inadequately (see Fung 1971) referred to as the Maxwell and the Voigt model Results of previous studies seem to favour the idea that the parallel elastic element in heart muscle is arranged in parallel with both the series elastic and the contractile element (For

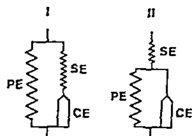


Fig 1 Mechanical models of heart muscle In the text the models are denoted model I (A) and model II (B) PE SE and CE indicate parallel elastic element series elastic element and contractile element respectively

references see reviews by Brady (1968) and Jewell and Blinks (1968)) The choice of muscle model is of great importance for determining the force velocity relation of the contractile element from data from the whole muscle (Hefner and Bowen 1967, Pollack 1970) Experiments to be described in this section were undertaken in order to see whether stiffness determinations (using small length changes) in active and in resting papillary muscles could provide further information about the location of the parallel elastic element

The stiffness of the *series elastic element* at different forces was determined by measuring the drop in force induced by a small decrease in length of a contracting papillary muscle kept at a length where resting force was low Such measurements can be performed with the series elastic element at various levels of force by imposing the length change at different times during an isometric contraction If the resting force of the preparation is low enough the influence of the parallel elasticity can be neglected and the measurements can be used to determine the stiffness of the series elastic component regardless of whether model I or II best represents the tissue It should be pointed out that this analytical approach for determining the stiffness force relation of the series elastic element implies that variation in the intensity of the active state does not substantially affect the compliance of this element a supposition supported by previous studies (Parmley and Sonnenblick 1967 Edman and Nilsson 1968)

According to model I, the stiffness of the *parallel elastic element* can be obtained in a corresponding way by making small releases during the diastolic interval at various resting forces and observing the concomitant fall in force In model II the length change of the whole preparation for a given change in resting force is the sum of the length changes in the parallel and the series elastic elements i e $(dL/dP)_M = (dL/dP)_{PE} + (dL/dP)_{SE}$ This relation can be rearranged as

$$(dP/dL)_{PE} = (dP/dL)_{SE}(dP/dL)_M/[(dP/dL)_{SE} - (dP/dL)_M] \quad (1)$$

where all (dP/dL) values refer to the same force (The symbols in this and following expressions are L = length T = time P = force subscripts M CE, SE and PE denote parameters associated with whole muscle contractile element series elastic element and parallel elastic element respectively) Thus by measuring the stiffness of the series elastic element (during muscle activity) and the stiffness of the entire preparation at various forces it is possible to deduce *the stiffness versus force relation* also for the parallel elastic component in model II

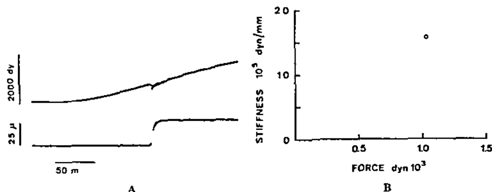


Fig 2 A Oscilloscope records showing stiffness determination of a contracting rabbit papillary muscle Upper trace force lower trace length change Lower end of force bar indicates zero force A drop in force is produced by releasing one end of the preparation to enable a small length change Stimulat on artefact visible Resting length of preparation 5 7 mm resting force 100 dyn Preparation no 7 of Table I

Fig 2 B Relation between active force and stiffness of a papillary muscle Release distance during stiffness determinations ○ 13 μ ● 29 μ □ 65 μ Resting length of preparation 5.2 mm resting force 100 dyn. Each point represents mean of at least two determinations Values corrected for stray compliance

A *The stiffness of the series elastic element*

The stiffness of papillary muscles during activity was measured as shown in Fig 2 A The resting force of the preparation was low during these determinations approximately 100 dyn therefore the influence of the parallel elastic element will not be considered in this section The stiffness was defined as $\Delta P/\Delta L$ where ΔP = fall in force induced by the length change ΔL. The force of the series elastic element corresponding to this stiffness i e the mean force of the preparation during the release is defined as $P_M - \frac{1}{2}\Delta P$ P_M being the force of the preparation at the start of the release The stiffness of the series elastic element was found to be independent of the release distance in the range 0 3 to 1 3 % of resting muscle length as demonstrated in Fig 2 B The mean ΔL value used in stiffness determinations in active preparations was 22 μ or 0 44 % of resting muscle length (*cf* Table I) At each of 5—10 different force levels on the rising phase of the isometric twitch 2 or more stiffness determinations were performed and the mean stiffness value was plotted against the corresponding force As previously found (Edman and Nilsson 1968) stiffness of the series elastic element increased recti linearly with force This relation can be described by the equation

$$\Delta P/\Delta L = K_A P + C_A \qquad (2)$$

in which K_A is the slope of the line and C_A its intercept with the ordinate

The symbol P in (2) denotes total force of the preparat on In the p eceed ng paper (Edman and Nilsson 1968 pag 215) an expression sim lar to (2) was used n wh ch P ndicated only acti e force

TABLE I *Stiffness constants of active and resting papillary muscles defined as K_A and K_R respectively in equation $\Delta P/\Delta L = K\ P+C$* All values corrected for a stray compliance of $20\mu/10^3$dyn

Exp no	Dimensions of muscle		Release distance during stiffness determinations		K_A mm^{-1}	K_R mm^{-1}	K_R-K_A mm^{-1}	P
	length[1]	diameter	active preparation	resting preparation (mean value)				
	mm	mm	μ	μ				
1	5 0	0 5	12	9	23 5[2](5)[3]	42 7(7)	19 2	< 0 1
2	4 5	0 8	29	15	23 3(5)	49 7(5)	26 5	< 0 05
3	5 2	0 5	25	9	11 6(8)	16 7(6)	5 1	< 0 [4]
4	6 1	0 5	30	27	8 5(6)	29 6(9)	21 1	< 0 01
5	4 8	0 9	19	16	9 0(10)	19 6(7)	10 5	< 0 01
6	3 5	0 6	11	7	20 1(8)	36 9(6)	16 8	< 0 1
7	5 7	0 4	26	18	10 7(8)	14 1(6)	3 4	< 0 1
mean ±SEM	5 0	0 6	22	14	15 2 ±2 6	29 9 ±5 2	14 7 ±3 2	

[1] Length of preparation in this table as well as in dimension of constant b of Hill s equation in Table II and in Fig 5–8 refer to the length at a resting force of 100 dyn
[2] Correlation coefficients for K_A and K_R 0 93±0 03 and 0 97±0 01 (mean±S E) respectively
[3] Numbers within brackets = n
[4] P denotes probability level of random difference between K_R and K_A

K_A values obtained in 7 expts calculated by means of the method of least squares are given in Table I The force extension curve of the series elastic element can be obtained by integrating (2) which gives

$$P_2 = \left(P_1 + \frac{C_A}{K_A}\right) e^{K_A(L_2-L_1)} - \frac{C_A}{K_A} \qquad (3)$$

where P_1 and P_2 represent force of the series elastic element at length L_1 and L_2 respectively With the mean K_A value of Table I it was calculated that in order to reach an isometric force of 1000 dyn from an initial level of 100 dyn the series elastic element had to be stretched 0 15 mm or 3 0 % of the muscle length

B *The stiffness of the parallel elastic element*

The stiffness of resting muscle was determined by using a procedure similar to that described for active preparations (Fig 3) The stiffness and the corresponding resting force were defined as $\Delta P/\Delta L$ and $P = P_{M_R} - \frac{1}{2}\ \Delta P$, P_{M_R} being resting muscle force at the start of the release As in active stiffness determination at least two measurements were carried out at each of 5–10 resting force levels The mean length change used in these experiments was 14 μ or 0 28 % of muscle length (The release distance could be varied within the range 0 2 to 1 1 % of resting muscle length without significant changes in the obtained stiffness values) The relation between $\Delta P/\Delta L$ and P was rectilinear within the range studied and could be fitted by the equation

$$\Delta P/\Delta L = K_R P + C_R \qquad (4)$$

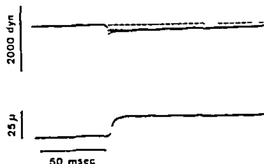
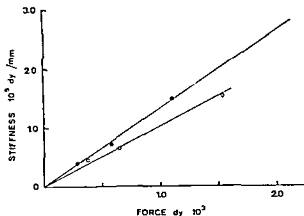


Fig 3 Oscilloscope records showing stiffness determination of a resting papillary muscle (same preparation as in Fig 2 A) Upper trace force lower trace length change Initial force level indicated by dotted line Note the slow increase in force after the length change



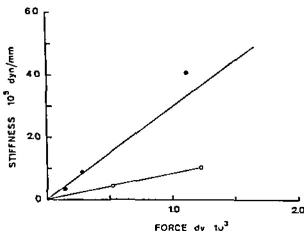


Fig 4 Stiffness force re t ons of two rabb t papillary muscles Symbols (○) active prepara tions (●) resting preparations Each point rep esents mean of at least two measurements Values corrected for stray compliance Slopes of lines calculated according to the method of least squares A exp no 7 and B exp no 4 of Table I

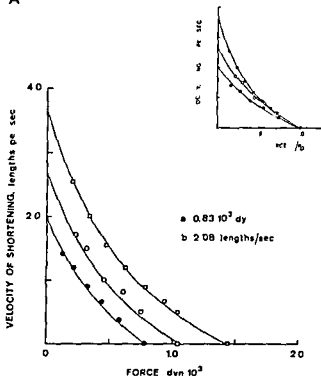
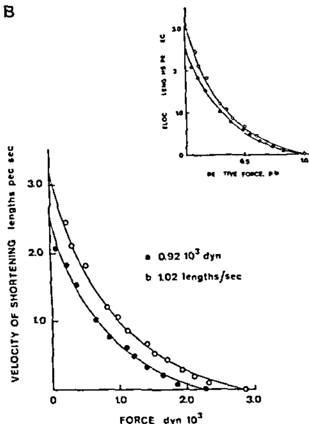


Fig 3 Force velocity curves of two rabbit papillary muscles measured at different lengths of the contractile element Determinations were made at the moment of the peak of the isometric twitch Resting force 50–100 dyn at all lengths from which releases were initiated Lengths of preparations before releases (mm) A □ 6 0 ○ 5 8 ● 5 6 B ○ 6 5 ● 6 0 Curves drawn according to Hill s equation constants a and b shown in figure Exp no 1 (A) and 5 (B) of Table II Insert figures show velocity of shortening as a function of relative force i e force at velocity measurement/active force at peak isometric twitch at the respective length In each force velocity curve in this and following figures variation in length of the contractile element between measurements with the smallest and the greatest load used was less than 2 0 / of resting muscle length For details of this calculation see Appendix II

where the subscript R denotes that the constants refer to resting muscle Table I gives K_R values obtained according to the method of least squares for the same preparations as were used for determinations of the series elastic stiffness Fig 4 illustrates the relation between force and stiffness for 2 preparations one with the smallest and the other with the greatest K_R/K_A ratio As is evident from Table I K_R is significantly greater than K_A which means that at any given force the compliance of the resting preparations is less than that of the active preparation (The constants C_R and C_A are both small and can be neglected except at very low forces) This finding indicates that the series elastic element cannot be located in series with both the contractile element and the parallel elastic element as in model II (cf eq 1) On the other hand according to model I the stiffness of the resting preparation equals the stiffness of the parallel elastic element, whereas the stiffness of the active preparation is the sum of the stiffness of the two elastic elements Thus the present results are fully compatible with a parallel elastic element located as in model I but not as in model II The calculations presented in the following section are based upon this conclusion

The present stiffness measurements of active preparations aimed at determining the series elastic stiffness were carried out at a low resting force (100 dyn) and the error introduced by neglecting the influence of the parallel elastic element upon these results is small (At a total muscle force of 1000 dyn and a resting force of 100 dyn the stiffness of the series elastic element is overestimated by 12 by ignoring the parallel elasticity This figure is based upon the mean K_A and K_R values of Table I)



II *Force velocity curves at different lengths of the contractile element*

A *Measurements at low resting forces* $\leqslant$ 100 dyn

The damped release method previously described (Edman and Nilsson 1968) was used in the force velocity determinations All releases for one force velocity curve were initiated at the same length of the preparation just prior to the peak of the isometric twitch In this way all velocity measurements could be made at the moment of the peak force which means that the peak force can be used as a value for P_0 in defining the force velocity curve Results of two experiments are shown in Fig 5 In both preparations alterations in muscle length caused approximately parallel changes in P_0 and V_{max} As the resting force was very small compared to the active force in these experiments the curves can be taken as representative for the contractile element itself In the inserts of Fig 5 the force at each velocity determination is given as a fraction of P_0 at the respective length After replotting the data in this way differences in extrapolated V_{max} between curves obtained at various lengths still exist indicating that these differences do not depend upon variations in P_0 Similar results were reached in 5 other preparations Table II shows data from all 7 expts in which two force velocity curves were determined at lengths corresponding to low resting forces

TABLE II P_0 (*maximum active isometric force*) *and constants a and b of force velocity curves determined with the damped release method* Releases were made from 2 lengths L_2 and L_1 both with a low resting force (< 100 dyn)

Exp no	P_0 at length L_1 and L_2 dyn × 10		Difference in length $L_2 - L_1$, % of L_2	Constants in Hill's equation	
	L_1	L_2		a dyn × 10	b lengths/sec
1	0 81	1 51	5	0 83	2 08
2	1.28	1 90	7	1 40	1 05
3	1 54	2 01	5	1 38	2 69
4	1 69	2 61	7	1 11	1 51
5	2 28	2 86	7	0 92	1 0?
6	2.72	2 80	2	0 96	0 98
7	1 63	2 11	15	1 13	2 04

B *Measurements at non negligible resting forces*

In order to make force velocity determinations of the contractile element at high resting forces the following procedure was used According to model I the shortening velocity of the contractile element during a contraction cycle is given by the following relation

$$-(dL/dT)_{CE} = \frac{(dP/dT)_M}{(dP/dL)_{SE}} - (dL/dT)_M \left(1 + \frac{(dP/dL)_{PE}}{(dP/dL)_{SE}}\right) \qquad (5)$$

The derivation of this equation is given by Hefner and Bowen (1967) and Pollack (1970) and more extensively by Fung (1970) During an isotonic contraction $(dP/dT)_M = 0$ thus the first expression on the right hand side of (5) is zero Therefore in order to calculate the shortening velocity of the contractile element during isotonic shortening of a preparation according to (5) it is necessary to know, in addition to the shortening velocity of the preparation the stiffness of the parallel and the series elastic components

In the present study stiffness values used in equation (5) are based upon stiffness measurements carried out as described in the preceding section The figures needed to calculate force velocity data of the contractile element according to equation (5) were obtained as follows $(dL/dT)_M$ was read directly from the electrically differentiated length signal on the oscilloscope tracing at the moment of the peak of the isometric twitch $(dP/dL)_{PE}$ and $(dP/dL)_{SE}$

In model I $$P_{SE} = P_{CE} = P_M - P_{PE} \qquad (6)$$

where both P_{PE} and P_{SE} are functions of the length and the stiffness of the respective element (*i.e* $P_{PE} = \int_{L_0}^{L_1} (dP/dL)_{PE}\, dL$ and $P_{SE} = \int_{L_0}^{L} (dP/dL)_{SE}\, dL$ L_1 being the actual length of the respective element and L_0 its length at zero force) At a given resting length (L_P of the preparation $P_{PE} = P_R$ (P_R = resting force of the preparation) When the length of the preparation is reduced during activity e g during a release experiment P_{PE} falls to a lower value on the length force curve of the parallel

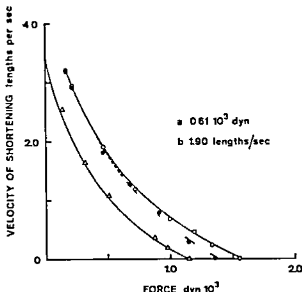


Fig 6 Force velocity curves of one rabbit papillary muscle obtained after releases from lengths shorter than that optimum for active force All data collected at the moment of peak isometric twitch The curve at the shortest length has been drawn according to Hill s equation with constants a and b as indicated Filled symbols show the calculated force velocity data of the contractile element at the longer muscle length For details of this calculat on see text Resting length and resting force of preparations before releases △ 3 5 mm and 100 dyn ○ 3 6 mm and 640 dyn

elastic element It should be noted that this force length curve of the parallel elastic element has a steeper slope than the passive force length relation of the preparation (see below section IV B and Fig 7 8 13) Thus at muscle lengths $L < L_R$

$$P_{PE} = P_R - \int_{L}^{L_R} (dP/dL)_{PE}\, dL \qquad (7)$$

The length of the preparation and therefore the length of the parallel elastic element at a given force velocity determination after a release was read from the oscilloscope tracing The force of the parallel ealstic element at this length was calculated from equation (7) using the K_R values of Table I The force across the series elastic element which equals the force of the contractile element is then obtained from equation (6) Finally the stiffness values for the two elastic elements at the moment of the force velocity measurement can be calculated from equations (2) and (4)

When st ffness determinations and force veloc ty measurements were performed on the same preparation the K values derived experimentally for this particular preparat on were used in the calculations For other preparations the mean K_A and K_R values of Table I were used

In 7 preparations force velocity curves of the contractile element at different resting forces were calculated as described above With increasing muscle length below the length optimum for active force V_{max} and P_O increased almost in parallel This is illustrated by results of one experiment shown in Fig 6 The full lines are drawn through the experimentally obtained force velocity measurements At the shorter length the force velocity curve of the contractile element is identical with that of the whole preparation fitted by Hill s equation with the constants a and b as shown in the figure At the longer leng h where releases were made from a high resting force the corresponding force velocity curve of the contractile element (dott d line) was calculated as described above

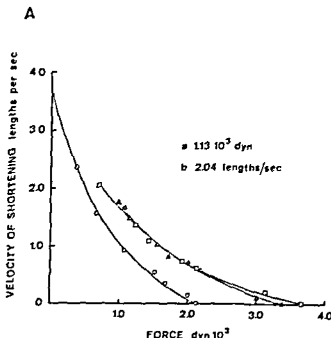
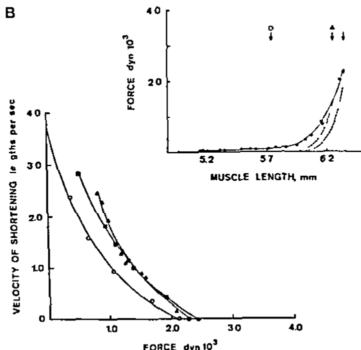


Fig 7 Force-velocity curves of one papillary muscle obtained at the moment of peak twitch force after releases from different muscle lengths

A. *Experimentally obtained force velocity curves of the whole preparation Curve through (o) values fitted according to Hill's equation with constant a and b as indicated other curves fitted by eye Resting lengths and resting forces corresponding to different curves are shown in insert of Fig 7 B*

When the length of a preparation was increased towards the apex of the active force length diagram the shape of the force velocity curve of the contractile element was slightly altered shortening velocities at low loads were affected more than the velocities at high loads by length changes Such a shift in the force velocity relation was observed in all the four experiments in which measurements were made near the muscle length optimum for active force Fig 7 illustrates results of one experiment The force velocity data of the contractile element at high resting forces have been calculated from force velocity measurements of the whole preparation as described above whereas at the length with a low resting force the force velocity curve of the whole preparation may be considered representative also for the contractile element

In the experiments described so far velocity measurements were carried out at only 2 or 3 lengths of each preparation and the force velocity curves obtained at great lengths were incomplete as the preparations were generally not released to loads less than the resting force In order to make velocity determinations over a wide range of muscle lengths the following type of experiment was performed on 3 preparations Releases were made at the peak of the isometric twitch from various lengths using the same isotonic load in all releases A relatively small load was chosen (approximately 20 % of maximum active force of the preparation) so that the length



B Force velocity curves of the contractile element of the same preparation as in Fig 7 A. Curve obtained at the shorter length (o) is identical in Fig 7 A and B (■) and (▲) represent force velocity data of the contractile element calculated from measurements on the whole preparation (□) and (△) of Fig 7 A respectively Insert figure shows resting force (●) of the preparation as a function of its length Arrows and large symbols indicate resting lengths from which releases for respective force velocity curves were initiated Dotted lines illustrate force extension curves of parallel elastic element for further information see text

change of the preparation during the first shortening phase after the release was great enough to make the parallel elastic force negligible at all velocity determinations (In order to calculate the fall in parallel elastic force with shortening a K_R value of 29 9 mm^{-1} was used *cf* Table I) Results of one experiment are shown in Fig 8 in which shortening velocity resting force and P_0-P (*i e* the active force at the respective length less the isotonic load) are plotted as functions of resting muscle length If the force velocity curve of the preparation obeys Hill s equation $(P+a)(V+b) = (P_0+a)b$ with the same constants a and b over the whole range of muscle lengths studied then $V = \frac{b}{P+a}(P_0-P)$ Thus V should change in proportion to P_0-P if the same isotonic load is used in all velocity measurements (*cf* Abbott and Wilkie 1953 Gordon Huxley and Julian 1966) As is evident from Fig 8 V changes slightly less than P_0-P in the rabbit papillary muscle However from this experiment and 2 others yielding similar results it can be concluded that the shortening velocity of the contractile element against a small load increases with elongation over the whole ascending section of the active force length curve

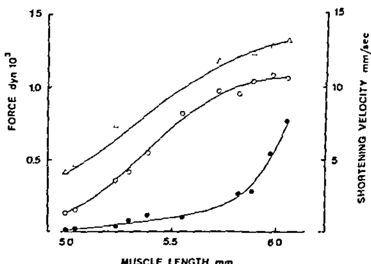


Fig 8 Isotonic shortening velocity (△) resting force (●) and P_0-P (○) of one preparation as functions of muscle length The isotonic shortening velocity was measured at a constant load (250 dyn) after releases from different lenghts P_0-P is defined as the maximum active force at respective length less the isotonic load Stimulation frequency 42 beats per min

III *Influence of muscle length on the time course of the active state*

The damped release method was used for studying the influence of muscle length on the time course of the active state In each of 6 preparations active state determinations were performed after releases from two different muscle lengths The isotonic load of the preparations was the same in the two series of measurements 100—200 dyn greater than the resting force existing at the longer muscle length All velocity data used for construction of one active state curve were collected at the same length of the contractile element after the series elastic element had adjusted itself to the load of the preparation (see Fig 1 Edman and Nilsson 1969) In some of these experiments the preparations were released from lengths where an appreciable resting force existed A fraction of the load during the isotonic shortening phase might therefore have resided across the parallel elastic element when the velocity measurements of a release curve were obtained However, in all experiments this parallel elastic force at the velocity determinations was less than 2 $\%$ of the isotonic load used (This statement is based on the presumption that the force of the parallel elastic element changes with muscle length according to equation 7 For the calculation a K_R value of 29 9 mm^{-1} was used *cf* Table I)

Fig 9 shows the results of one experiment The time course of the active state obtained after releases from the greater muscle length is prolonged the main effect being a delay in the onset of the decay phase In some experiments including that of Fig 9 the rate of decay was slightly lower at the greater muscle length As is evident from Table III which sumarizes the results of all 6 expts the time from stimulus to

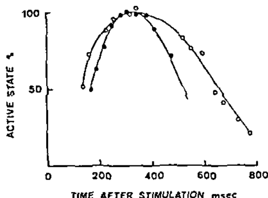


Fig 9 Time course of active state at two different lengths of the contractile element obtained by means of the damped release method Rel ases were initiated from muscle lengths 5 9 mm (●) and 7 3 mm (○) and velocity measurements were performed at muscle lengths 5 8 mm and 7 1 mm respectively Exp no 3 of Table III

60 % decay of the active state was longer the longer the initial muscle length Each per cent change of resting muscle length altered the time to 50 % decay by 6–17 ms In the experiment illustrated in Fig 9 the active state reached 60 % of its maximum value at a slightly earlier time at the greater length However in this respect, no significant effect of muscle length was found see Table III

IV *Influence of muscle viscosity*

Several previous studies have demonstrated viscous properties of isolated myocardial preparations (Lundin 1944 Hoffman Bassett and Bartelstone 1968 Rumberger and Schwartz 1969, Rumberger 1970) In the present study a slight viscous component was also evident in stiffness measurements of resting papillary muscles This was shown by the redevelopment of force following the initial force drop induced by a small decrease in length However as illustrated in Fig 3 this secondary increase in force after a length change had a slow time course (In tracings of Fig 3 it can be seen

TABLE III *Time course of active state at differe t len ths of the contractile element mea ured by means of the damped release method* Releases were made from two muscle lengths L_1 and L_2 using th same isotonic load in both series of releases In each series all shortening elocit es were determined at the same length of the preparation

Exp no	Rest ng force at L_1 dyn×10	L_2-L of L_1	Time from stimulus to 60 rise of act ve state			T me from stimulus to 60 decay of active state		
			L_2	L_1	Difference msec/length change	L_2	L_1	Difference msec/length change
1	0 23	4	130	130	0	560	510	13
2	0 16	6	140	140	0	380	340	7
3	0 58	19	140	180	2	670	500	6
4	0 58	13	180	210	2	560	470	7
5	0 77	6	190	190	0	6 0	570	17
6	0 36	4	160	150	−3	500	440	15

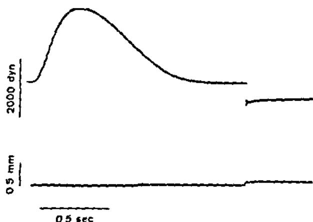


Fig 10 Release records of resting papillary muscle following an active contraction Upper trace force lower trace, length change Release distance 75 μ Resting length of preparation before release 7 3 mm

that 50 ms after the length change the preparation had regained approximately 20 % of the force dropped during the release) The magnitude and the time course of such force changes which might be attributable to muscle viscosity were most clearly seen on oscilloscope records with a slow time base Fig 10 shows release curves of one preparation, in which the magnitude of the release was slightly greater (75 μ) than in ordinary stiffness measurements It is evident that the change of force *after* the release is small compared with the force drop *during* the release These results suggest that only a small part of the dynamic stiffness of resting preparations measured as described in the present study is attributable to viscocity effects Moreover, the time course of such viscocity effects seems slow enough to justify the presumption that they do not influence force velocity measurements as performed in the experiments presented in preceding sections In order to examine this question furth r length changes in active and in resting papillary muscles following damped releases were compared Fig 11 A shows release tracings of a resting papillary muscle The preparation had been prestretched to a length corresponding to a resting force of 3400 dyn and was released to a force of 100 dyn The length of the preparation after the release changes in two phases as would be expected of a visco elastic unit (lower trace) However the length change during the second shortening phase is only 20 % of that during the first and the shortening velocity during the second phase is negligible compared with the velocity during the first shortening phase (upper trace) This is in contrast to the situation in the same preparation during an active contraction when a similar fall in force is initiated by releasing the preparation at the peak of the isometric twitch (see Fig 11 B) The total length change during the second shortening phase in this case is 2 8 times greater than during the first phase and the velocity at the beginning of the second shortening phase is 34 % of the maximum velocity of the first phase It has been shown that in frog myocardium the viscosity is of the same order in resting and in active muscle (Lundin 1944) Assuming that to be so also in mammalian myocardium only a very small

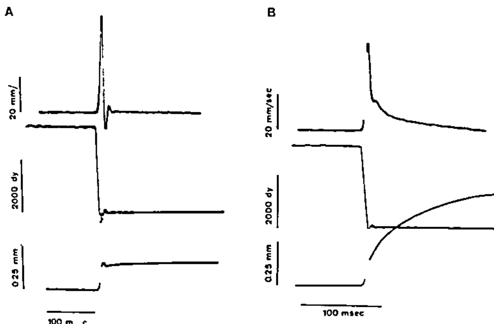


Fig 11 A D mped release of a resting papillary muscle Final force after the release 100 dyn Curves from above velocity of shortening force and shortening (Downstroke of force trace has been redrawn) Note that the velocity trace falls from its maximum value to below the zero level without any hump of the curve

B Damped release of the same preparation as in Fig 11 A initiated from the peak of the isometric t vitch Re ting force of the preparation 100 dyn force level after the release 100 dyn Curves from above velocity of shortening force and shortening (Downstroke of force trace has been redrawn) Note the appearance of 2 d stinct phases in both shortening and shortening velocity traces

error is introduced by neglecting velocity components due to viscocity transients after releases This means that the econd shortening phase in Fig 11 B can be considered representative of the shortening velocity of the contractile component alone as previously discussed (Edman and Nilsson 1968)

The dynamic stiffness of a resting papillary muscle measured at a given force as described previously in this report was found to exceed its static stiffness as defined by the slope of the passive length force curve This is demonstrated in Fig 12 which shows for the same preparation both the static length force relation of a preparation (full line) and the extension force curve of its parallel elastic element obtained by dynamic measurements (dotted line) The latter was obtained by integration of equation (4) using the experimental data of this preparation (no 7 in Table I) Evidently the force rises more steeply with increasing length in the latter than in the former curve This accords with the idea expressed above of (a) viscous component(s) located in parallel with the parallel elastic element

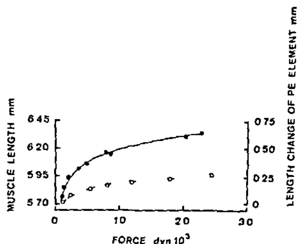


Fig 12 Resting length force diagram of one papillary muscle (●) and extension force curve of parallel elastic element of the same preparation (○) The latter was calculated by using the experimentally obtained constant K_R of this preparation no 7 of Table I Both curves start at a force level of 100 dyn

Discussion

Stiffness determinations and models of muscle contraction

In the present study two simple models of muscle were considered and the choice between them was based upon stiffness determinations in actively contracting and in resting papillary muscles The results indicated that the major portion of the parallel elastic element must be located in parallel with both the contractile element and the series element as in model I This model should no doubt be regarded as an over simplification which none the less may be of use in studying the dynamic properties of the contractile element No viscous component was considered when calculating force velocity data of the contractile element from velocity mesaurements of the whole preparation This seems justified as velocity measurements after a release were only slightly influenced by viscocity effects Results of stiffness determinations of the series elastic element in the present study were in accordance with values obtained previously with the same technique (Fig 7 in Edman and Nilsson 1968) taking into account that the previous data also included the stray compliance of the recording device Considerably lower stiffness values for the series elastic element have been obtained by means of the standard quick release technique (Abbott and Mommaerts 1959 Parmly and Sonnenblick 1967 Hefner and Bowen 1967) This discrepancy in results can to some extent be explained by the length oscillation that appears at the end of the first rapid shortening phase following an undamped quick release (cf Fig 4 of Abbott and Mommaerts 1959 Fig 3 of Hefner and Bowen 1967, Edman and Nilsson 1972) Such an oscillation may lead to an overestimation of the length change of the elastic element after a drop in force

In order to compare the elasticity of different tissues compliance can be defined as the percentage increase in length necessary to produce an e fold increase in tissue force assuming that the stress strain relations of these tissues are exponential (Brady 1968) Stated thus the dynamic compliance of the series elastic and the parallel

elastic element of papillary muscles used in this study are 1 3 % and 0 7 % of resting muscle length respectively (These figures were obtained by using the mean values of resting muscle length K_A and K_B of Table 1) By comparison the dynamic compliance of the series elastic element in frog sartorius is about 1 % at 2 C (Jewell and Wilkie 1958 p 521) Data concerning dynamic stiffness in resting skeletal muscle comparable with those reported above for resting papillary muscle seem to be lacking It is of interest however that the finding of a much greater dynamic than static stiffness of resting mammalian myocardium in the present study (section IV of Results) is in line with results of previous studies on both single skeletal muscle fibres of the frog (Buchtal Kaiser and Rosenfalck 1951) and on frog cardiac muscle (Lundin 1944)

The force velocity curve

In order to relate a force velocity curve to a given functional state of the contractile element it is necessary to perform all measurements for one curve at the same intensity of the active state and at the same length of the contractile element (Edman and Nilsson 1968 1972) In the present study these criteria were fulfilled by measuring the velocity of the preparation against different loads at the moment corresponding to the peak of the isometric force When necessary the data so obtained were corrected for the influence of the parallel elastic element in order to derive the force velocity curve of the contractile unit alone Alterations in length of the muscle at lengths shorter than the optimum for active force caused V_{max} and P_O of the force velocity curves of the contractile element to change to approximately the same degree whereas at greater muscle lengths the extrapolated V_{max} changes more than P_O The finding that V_{max} of myocardial force velocity curves is length dependent accords with the results of Noble Bowen and Hefner (1969) but it is in apparent contrast to the conclusion reached by Sonnenblick (1962) However as pointed out previously (Edman and Nilsson 1968 Pollack 1970) afterloaded force velocity curves as considered in Sonnenblick s study cannot be taken to represent the properties of the contractile element at a constant intensity of the active state Further more in the study of Sonnenblick (1962) the influence of the parallel elastic element was not considered

Studies on papillary muscle of cat (Spotnitz Sonnenblick and Spiro 1966) and of rat (Grimm and Whitehorn 1968 Grimm et al 1970) have shown that maximum active force in myocardium is produced in the sarcomere region 2 0—2 2 μ i e at the region of maximum overlap between the thin and the thick filaments If these conditions also apply to papillary muscles used in the present study it means that at sarcomere lengths shorter than those giving maximum overlap between the two sets of filaments V_{max} and P_O change approximately in parallel whereas at greater lengths only V_{max} is altered (No force velocity determinations were performed at muscle lengths great enough to give an appreciable reduction of P_O of the contractile element)

It should be pointed out however, that the quantitative relation between sarcomere length and active force is difficult to assess on the basis of previous studies on myocardial preparations (Spotnitz, Sonnenblick and Spiro 1966 Grimm and Whitehorn 1968 Grimm *et al* 1970) As heart cells are not maximally activated during an ordinary contraction cycle, the active force of a myocardial preparation is influenced not only by changes in the degree of overlap between the A and I filaments, but also by any factor changing the intensity and/or the duration of the active state As discussed below, length changes *per se* will affect the duration of the active state and hence also the relation between peak isometric force and muscle length

It is of interest to compare the results of the present study with previous findings on skeletal muscle The parallel change in V_{max} and P_0 below the length of maximum force accords with previous findings obtained on whole sartorius muscle of the frog (Abbott and Wilkie 1953 Matsumoto 1967) Furthermore in single muscle fibres of the frog it has been demonstrated that in the sarcomere length region between 1 7 and 2 0 μ the tetanic force and the shortening velocity against a light load decreases approximately to the same extent and at lengths shorter than 1 7 μ the shortening velocity decreases more than the isometric force (Gordon Huxley and Julian 1966) In the same study, it was also shown that the tetanic force reaches a plateau in the sarcomere region 2 0–2 25 μ and decreases at greater lengths whereas lightly loaded shortening velocity remains constant or slightly increases at lengths greater than 2 0 μ In accordance with the sliding filament hypothesis, the decrease in force and shortening velocity at sarcomere lengths below 2 0 μ was ascribed to the meeting of thin filaments in the centre of the sarcomere with consequent increase in internal resistance to the sliding movement An additional factor that can cause decrease in both active force and shortening velocities at low loads is a reduction in the degree of activation of the contractile system at short sarcomere spacings as observed in single skeletal muscle fibres (Taylor and Rudel 1970, Rudel and Taylor 1971) Thus on the basis of results presented in the present study and in previous investigations on myocardium and on skeletal muscle it seems feasible to conclude that active force and shortening velocity in the two types of muscles vary with sarcomere length in basically the same manner

The time course of the active state

The results of the present study indicate that the duration of the active state in myocardial cells increases with increasing length of the cells and that the effect is due mainly to a later onset of the decay phase of the active state As myocardial cells are mechanically unsaturated this increased duration of the active state will contribute to the enhancement of peak twitch force occurring when myocardial preparations such as papillary muscles are elongated No explanation for the length dependence of the time course of the active state is available at present However the possibility that the observed changes are governed by alterations of the time course of the myocardial action potential can be ruled out Previous studies (Dudel and Trautwein 1954 Penefsky and Hoffman 1963 Gennser and Nilsson 1968) have

shown that the length status of myocardial preparations does not affect the duration of the action potential in heart cells if excessive stretch is avoided

The length dependence of the time course of the active state in myocardial cells is in accordance with previous studies on skeletal muscle showing that the duration of mechanical activity in frog sartorius (Ritchie 1954) and in isolated semitendinosus fibres of the frog (Edman and Kiessling 1971) is smaller the shorter the muscle length

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Appendix

I *Calculation of natural frequency of the recording system*

A. *Natural frequency in the absence of damping of the lever movements*

Two different isotonic levers (I and II) were used during the course of this study both with a static friction of less than 2 mg The total equivalent mass of the moving systems (*i.e* the sum of the equivalent masses of the respective lever the steel wire connecting the lever to the preparation and the loading spring) was 100 mg and 150 mg for lever I and II respectively (Equivalent mass was determined by measuring the acceleration produced by a given force) The total stray compliance of the length recording system was 20 μ/10 dyn (see Methods)

The free resonance frequency of the recording system can be stated as $f = \frac{1}{2\pi}\sqrt{\frac{K}{m}}$ Hz where K is the force constant of the system and m is its equivalent mass Inserting into this expression the values for K and m given above f for lever I and II becomes 352 and 288 Hz respectively

B *Natural frequency in the presence of damping of the lever movements*

The equivalent mass of the recording device increased when the movements of the lever were damped The damped motion of the lever can be described as

$$F - \mu dL/dT = m\, d^2L/dT^2 \qquad (1)$$

where F is the force at the point of attachement of the steel wire and $\mu dL/dT$ is the damping force resisting the movement of the peg in the silicon oil

In order to determine the coefficient μ a spring was placed between the isotonic lever (whose position was fixed by means of a releasable catch) and the RCA force transducer which measured the force of the spring The catch was released and the lever was accelerated by the force of the spring When a constant velocity of the lever was attained $d^2L/dT^2 = 0$ and $\mu = \frac{F}{dL/dT}$ In one typical experiment with lever I (which had an equivalent mass in air of 100 mg) μ was found to be 24 dyn s/cm with a damping fluid of viscosity 6 000 Cs During this release the maximum acceleration of the lever was 1 09 10 cm/s when the velocity was 4 0 cm/s The corresponding net force (*i e* the force of the spring 288 dyn less the damping force) was 193 dyn. If these figures for acceleration and force are used the equivalent mass of the mechanical system with the damping arrangement is 177 mg A similar determination for lever II (which had an equivalent mass in air of 150 mg) gave a figure for μ of 55 dyn s/cm and an equivalent mass of 250 mg in the presence of the damping system

The force between the isotonic lever and the force transducer during an experiment can be defined as

$$F = -K L \qquad (2)$$

where L = deviation of the system from its equilibrium position and K = the force constant of the system Combining (1) and (2) gives

$$m\frac{d^2L}{dT^2} + \mu\, dL/dT + K L = 0 \qquad (3)$$

which is the equation for damped harmonic motion If $\frac{\mu}{2m} < \sqrt{\frac{k}{m}}$ as in the present case the solution to (3) becomes (Reddick and Miller 1955)

$$L_T = L_0 \, e^{-\mu T/m} \cos(\omega' T + \delta) \tag{4}$$

in which L_0 and L_T are the deviations of the lever tip from its equilibrium position at times zero and T respectively and

$$\omega = \sqrt{\frac{k}{m} - \left(\frac{\mu}{2m}\right)} \tag{5}$$

Inserting the values of k m and μ given above for each lever into (5) the calculated natural frequency $\left(f = \frac{\omega}{2\pi}\right)$ of lever I and II is 265 and 223 Hz respectively

II *Calculation of the length change of the contractile element during a damped release* The shortening velocity of the contractile element is assumed to be instantaneously adjusted according to the force velocity relation of the element The preparation is released at the peak of the isometric twitch at a muscle length corresponding to a low resting force The release starts at T_0 and the first, rapid shortening phase of the preparation ends at T_1 P_0 and P_1 denote the force of the contractile element at these respective times As a first approximation it can be assumed that the force of the preparation falls linearly with time in the release interval $T_0 - T_1$ the force $P(T)$ at time T in this interval can be stated as

$$P(T) = \frac{P_0 - P_1}{T_0 - T_1}\,(T - T_0) + P_0 \tag{1}$$

The force velocity relation of the contractile element can be written

$$-dL/dT = \frac{P_0 + a}{P + a}\,b - b \tag{2}$$

where P_0 = force at peak isometric twitch a and b the constants in Hill equation

Combining (1) and (2) and integrating gives the length change of the contractile element from time T_0 to T

$$L(T) - L(T_0) = -\int_{T_0}^{T} (dL/dT)\, dT' = -\int_{T}^{T} \left(\frac{P_0 + a}{P(T) + a}\,b - b\right) dT \tag{3}$$

Integration of (3) from time T_0 to T_1 yields the total length change of the contractile system during the first shortening phase after the release

$$L(T_1) - L(T_0) = \frac{P_0 + a}{P_0 - P_1}\,(T_1 - T_0)b\,[\ln(P_1 + a) - \ln(P_0 + a)] + b(T_1 - T_0)$$

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Acta physiol scand 1972 85 24—32

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Potentiation of the Gastric Secretory Response to Sham Feeding in Dogs by Infusions of Gastrin and Pentagastrin[1]

By

LARS SJODIN

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Abstract

SJODIN L *Potentiation of the gastric secretory response to sham feeding in dogs by infusions of gastrin and pentagastrin* Acta physiol scand 1972 85 24—32

The gastric acid secretory response to sham feeding in dogs provided with esophagostomies and Pavlov pouches was related to the length of the sham feeding period. In antrectomized dogs the response even to prolonged sham feeding (30 min) was small. When a small supra threshold dose (0.30 μg/kg/h) of synthetic human gastrin I or pentagastrin was infused these dogs responded markedly to a short period of sham feeding (1 min). When the sham feeding period was prolonged to 10 min subthreshold amounts of gastrin and pentagastrin (0.06 μg/kg/h or less) sufficed to enhance the sham feeding response. The enhancement fulfilled the criterion proposed by Gaddum for true potentiation. The pepsin output was also increased when the sham feeding period was prolonged. In the antrectomized dogs pepsin output after sham feeding was not significantly changed when the dose of gastrin infused was increased from 0.06 to 0.30 μg/kg/h or when the dose of pentagastrin was increased from 0.30 to 1.20 μg/kg/h

Vagal stimulation activates the secretion of gastric acid by direct action on the parietal cells and by release of gastrin from the pyloric antrum (Uvnas 1942, Olbe 1963). This concept is based on observations that acid responses to vagal stimulation elicited for example by sham feeding are greatly decreased by antrectomy (Straaten 1933, Uvnas 1942, Olbe 1964) and that preantrectomy responses to vagal stimulation can be restored by infusion of subthreshold amounts of crude gastrin preparations (Uvnas 1942, Olbe 1964). These findings also indicate that vagal impulses and gastrin act synergistically on the parietal cells. Further evidence for a vagal release of gastrin has been obtained by Fyro (1967) who found that electrical stimulation of the vagi reduced the gastrin activity of the antral mucosa in cats. Using radio-immunological techniques increased blood levels of gastrin have recently been demonstrated in dogs after sham feeding (Nilsson *et al* in press) and in humans during insulin hypoglycemia (Korman, Soveny and Hansky 1971).

Sham feeding like other vagal stimuli evokes the secretion of gastric juice rich in pepsin. Infusion of suprathreshold amounts of crude gastrin has been reported to

[1] Parts of this investigation were presented at the Nobel Symposium XVI—Frontiers in Gastro-intestinal Hormone Research—Stockholm 1970

reduce vagally induced pepsin output (Olbe Ridley and Uvnas 1968 Vagne and Grossman 1969)

The present investigation was undertaken to study the influence of pure gastrin and pentagastrin on gastric acid and pepsin secretion in response to sham feeding in antrectomized Pavlov pouch dogs Since graded responses can be obtained by varying the period of sham feeding (Preshaw and Webster 1967) the effects of gastrin and pentagastrin on the secretory responses to various periods of sham feeding were studied

Methods

Surgical procedures 9 mongrel dogs weighing 12—26 kg selected for their good appetite were prepared with esophagostomies according to the method described by Olbe (1959) In addition innervated gastric fundic pouches were constructed by a method slightly modified after Thomas (1947) In 4 dogs the antrum was resected together with 2—3 cm of the distal part of the corpus and the duodenal bulb Before the antrectomy the antrumcorpus border was visualized by the pH paper indicator technique (Andersson 1960 Olbe 1963) The completeness of the antrectomy was confirmed by histological examination Gastrointestinal continuity was restored by gastrojejunostomy At the same operation a gastric cannula (inner diameter 2 cm) was inserted into the most dependent part of the main stomach After each operation the dogs were normally allowed a period of 3 weeks for recovery

Experimental procedures The dogs were deprived of food but not water for 18—20 h before the start of the experiments No dog was used more than thrice a week Basal secretion was recorded for 1 h Samples were collected for 15 min or 1 h periods from the pouches and for 1 h periods from the main stomachs The volume was measured and the acidity determined by titration with 0 01 N NaOH with phenolphthalein as indicator Pepsin concentration was determined by the hemoglobin substrate method (Bucher Grossman and Ivy 1945) and the results are expressed in pepsin units

The dogs were sham fed for various periods Immediately before sham feeding the esophageal cannula was opened and a piece of gauze was tied tightly around the esophagus below the cannula to prevent food from passing down to the stomach The dogs were sham fed minced meat Food was never observed to leave the gastric cannula after sham feeding The secretory response was recorded for 3 h after sham feeding In some experiments 0 15 M NaCl or saline solutions of gastrin and pentagastrin in various concentrations were infused intravenously at a constant rate by a peristaltic pump (Harvard Apparatus Co Dover Mass USA) calibrated to deliver 25—30 ml/h The infusions were started 2 h before sham feeding and continued throughout the experiments In other experiments graded doses of pentagastrin were infused The dose was doubled every hour in order to obtain a dose response curve for pentagastrin The output for each dose has been calculated from the mean of the last two 15 min periods at each dose level

3 main series of experiments were performed

Series A 6 dogs with intact antrum were sham fed for 1 10 and 30 min

Series B 3 antrectomized dogs with open gastric cannulae were sham fed for 1 and 30 min They were also sham fed for 1 min during concomitant pentagastrin infusion 1 of the dogs was also sham fed for 10 min with and without infusion of pentagastrin

Series C 2 antrectomized dogs were sham fed for 1 and 10 min with and without concomitant infusion of synthetic human gastrin I

Test substances Pentagastrin (Peptavlon®) kindly supplied by AB Scanmeda Gothenburg Sweden

Synthetic human gastrin I kindly supplied by ICI Pharmaceuticals Division Alderley Park England

Statistical evaluation of data The results are mostly expressed as means ±S E However in 2 tables (Table III and IV) the ranges are given in place of the S E because of the low number of observations Only the results of the secretion of the pouches are presented in this paper The t test for unpaired values was used in the statistical analysis of differences in responses to various periods of sham feeding or to various doses of pentagastrin Snedecor and Cochran 1967) The differences were determined individually for each dog The probabilities of difference for groups of dogs were obtained by combining the probabilities for the individual dogs (Fisher 1948)

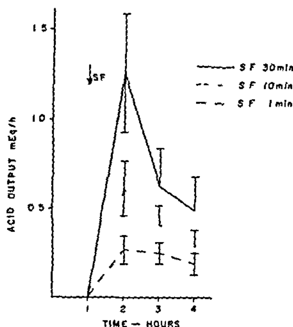


Fig 1 Acid outputs for 3 h after 1 10 and 30 min of sham feeding (SF) in 6 Pavlov pouch dogs Each point represents the mean of 4—6 expts on each dog In this and the following figures vertical bars show the S F

Results

Secretion of acid

Series A The 3 h acid response to sham feeding was increased as the period of sham feeding was increased from 1 to 30 min ($p < 0\,001$) The peak hourly acid output increased correspondingly (Fig 1)

Series B The acid responses to 1 and 30 min of sham feeding were small in 3 antrectomized Pavlov pouch dogs with open gastric cannulae (Table I) Table *II* and Fig 2 illustrate that 0 30—1 20 μg of pentagastrin/kg/h markedly enhanced the acid response to 1 min of sham feeding whereas 0 06 μg/kg/h had only a small effect The secretory responses to graded doses of pentagastrin alone in the same dogs are given in Fig 3

TABLE I Acid responses to 1 and 30 min of sham feeding in 3 antrectomized Pavlov pouch dogs with open gastric cannulae

Dog	Number of expts	Duration of sham feeding min	Acid output meq/3 h
D	4	1	0 02 ± 0 01
	3	30	0 17 ± 0 13
E	4	1	0 10 ± 0 07
	3	30	0 51 ± 0 24
F	4	1	0 01 ± 0 04
	4	30	0 08 ± 0 08

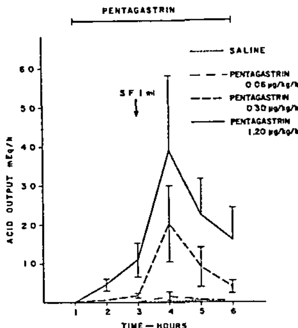


Fig 2 Acid responses to 1 min of sham feeding (SF) in 3 antrectomized Pavlov pouch dogs (D E and F) with open gastric cannulae during concomitant infusions of 0 15 M NaCl or pentagastrin Infusions were started 2 h before sham feeding and continued throughout the experiments Each point represents the mean of 3—4 expts on each dog

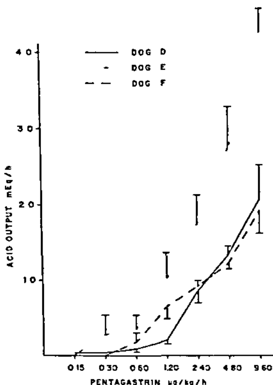


Fig 3 Acid responses to graded doses of pentagastrin in 3 antrectomized Pavlov dogs (D E and F) with open gastric cannulae Each point represents the mean of 3 expts on one dog

TABLE II Acid output for 1 and 3 h after 1 min of sham feeding (SF) in 3 antrectomized Pavlov pouch dogs with open gastric cannulae during concomitant infusions of 0.15 M NaCl or various doses of pentagastrin Acid outputs during infusion of saline and pentagastrin alone (2nd h of constant infusion) before sham feeding are also given

Dog	Number of expts	Dose of pentagastrin µg/kg/h	Acid output before SF meq/h	Acid output after SF (1st h) meq/h	Acid output after SF (3 h) meq/3 h
D	4	0	0.00±0.00	0.01±0.00	0.02±0.01
	3	0.06	0.00±0.00	0.40±0.33	0.71±0.61
	4	0.30	0.19±0.06	1.73±0.19	2.64±0.33
	4	1.20	0.55±0.14	1.68±0.13	3.42±0.10
E	4	0	0.00±0.00	0.01±0.00	0.10±0.07
	3	0.06	0.01±0.01	0.12±0.05	0.22±0.11
	4	0.30	0.28±0.19	3.89±0.97	6.54±1.48
	3	1.20	1.94±0.06	7.60±0.88	14.98±1.93
F	4	0	0.00±0.00	0.01±0.00	0.04±0.04
	3	0.06	0.00±0.00	0.00±0.00	0.00±0.00
	3	0.30	0.10±0.09	0.50±0.12	0.98±0.25
	3	1.20	0.81±0.05	2.34±0.07	4.88±0.65

TABLE III Acid responses to 10 min of sham feeding in 1 antrectomized Pavlov pouch dog with open gastric cannula (Dog F) during concomitant infusions of 0.15 M NaCl or various doses of pentagastrin

Number of expts	Dose of pentagastrin µg/kg/h	Acid output meq/3 h	
		Mean	Range
2	0	0.48	0.13—0.83
2	0.012	1.40	1.08—1.71
2	0.060	3.51	2.10—4.92
2	0.300	5.92	4.42—7.41

TABLE IV Acid responses to 1 and 10 min of sham feeding in 2 antrectomized Pavlov pouch dogs with open gastric cannulae during concomitant infusions of 0.15 M NaCl or various doses of gastrin Acid outputs during infusion of saline and gastrin alone (2nd h of constant infusion) before sham feeding (SF) are also given

Dog	Number of expts	Duration of sham feeding min	Dose of gastrin µg/kg/h	Acid output before SF meq/h		Acid output after SF meq/3 h	
				Mean	Range	Mean	Range
F	7	1	0	0.01	0.00—0.07	0.08	0.00—0.24
	2	1	0.06	0.00	0.00—0.00	0.22	0.18—0.25
	2	1	0.30	0.75	0.40—1.10	6.11	3.49—8.73
	2	10	0	0.01	0.01—0.01	0.48	0.13—0.83
	2	10	0.06	0.18	0.12—0.24	4.09	3.77—4.41
G	4	1	0	0.01	0.00—0.02	0.04	0.02—0.09
	2	1	0.06	0.05	0.03—0.06	0.32	0.14—0.49
	3	1	0.30	0.52	0.34—0.83	4.75	2.16—8.33
	5	10	0	0.01	0.00—0.03	0.15	0.03—0.30
	4	10	0.06	0.03	0.01—0.05	1.85	1.00—2.39

TABLE V Acid responses to various periods of sham feeding in 1 Pavlov pouch dog before resection of the pyloric region (Dog G) At this stage the dog had no gastric cannula

Number of expts	Duration of sham feeding min	Acid output meq/3 h
4	1	0 45±0 09
4	10	0 96±0 23
4	30	4 69±0 53

TABLE VI Mean pepsin responses (pepsin units PU[Hb]) to 1 10 and 30 min of sham feeding in 6 Pavlov pouch dogs 3—6 expts for each sham feeding period on each dog

Period of sham feeding min	Pepsin output PU[Hb]/10 /3 h	Significance of change
1	3300± 900	———
10	4600± 900	p<0 05
30	6900±1700	p<0 01

TABLE VII Mean pepsin responses (pepsin units PU[Hb]) to 1 min of sham feeding in antrectomized Pavlov pouch dogs with open gastric cannulae during concomitant infusions of pentagastrin (3 expts for each dose on each of 3 dogs D E and F) and gastrin (1—3 expts for each dose on each of 2 dogs F and G)

Number of expts	Dose of pentagastrin µg/kg/h	Dose of gastrin µg/kg/h	Pepsin output PU[Hb]/10 /3 h	Significance of change
9	0 30	0	1200± 300	———
9	1 20	0	3400±1500	p>0 05
2	0	0 06	1800± 500	———
5	0	0 30	2900±1100	———

1 of the dogs was later sham fed for 10 min with and without concomitant infusions of pentagastrin Table III illustrates that doses as low as 0 012 µg/kg/h were able to enhance the acid response to this period of sham feeding

Series C 2 antrectomized dogs were sham fed for 1 min with and without infusion of gastrin 0 30 µg/kg/h markedly elevated the acid response to sham feeding (Table IV) This dose given alone induced a small to moderate secretion (Table IV)

When the sham feeding was prolonged to 10 min in these dogs a lower dose of gastrin 0 06 µg/kg/h also markedly enhanced the acid response to sham feeding (Table IV) The secretory responses to 1 10 and 30 min of sham feeding in 1 of the dogs before antrectomy are given for comparison (Table V)

Secretion of pepsin

Series A The 3 h pepsin output was increased when the period of ham eeding was prolonged from 1 to 30 min ($p < 0\ 01$) as shown in Table VI

Series B. The mean pepsin output after 1 min of sham feeding tended to increase in 3 antrectomized dogs when the dose of pentagastrin was changed from 0.30 to 1.20 μg/kg/h (Table VII).

Series C. There was a tendency towards elevated pepsin responses after 1 min of sham feeding in 2 antrectomized dogs when the amount of gastrin was increased from 0.06 to 0.30 μg/kg/h (Table VII).

Discussion

Secretion of acid. The present results confirm that graded gastric acid responses can be obtained when the period of sham feeding is varied (Preshaw and Webster 1967, Nilsson 1969). The relation between duration of sham feeding and acid response seems to be the same whether the acid from the main stomach is diverted from the antroduodenal region by a gastric fistula (Preshaw and Webster 1967), partially diverted by isolation of the duodenal bulb combined with a gastrojejunostomy (Nilsson 1969) or not diverted at all as in the present study.

Resection of the antrum changed the relation between duration of sham feeding and acid response. Even prolonged sham feeding (30 min) did not induce any marked secretion in the antrectomized dogs in the present study. This is to some extent at variance with findings recently reported by Preshaw (1970). He found that antrectomized gastric fistula dogs gave a substantial response to 100 min of sham feeding. However, also in the study of Preshaw prolonged sham feeding (30—100 min) resulted in secretory responses which were far less after than before, antrectomy.

When 0.30 μg/kg/h of pentagastrin, producing by itself a barely significant secretion, was infused, marked secretory responses were obtained in the antrectomized dogs to 1 min of sham feeding. Only with severalfold up to more than 16 times higher doses of pentagastrin given alone did the secretion attain the same level as for the combination of 0.30 μg/kg/h and 1 min of sham feeding. Thus the synergism between these two stimuli in antrectomized dogs fulfils the criterion for true potentiation proposed by Gaddum (Gillespie and Grossman 1964): the response to the combination of pentagastrin and sham feeding was greater than the maximal response to sham feeding alone in antrectomized dogs and greater than the response obtained by doubling the doses of pentagastrin used.

When the sham feeding period was prolonged to 10 min, smaller doses of pentagastrin sufficed to potentiate the sham feeding effect. The smallest dose that effectively enhanced the response to 10 min of sham feeding was probably less than 0.2 nanogram/kg/min. The effects of gastrin were about the same as those of pentagastrin. The response to 1 min of sham feeding was markedly enhanced by a suprathreshold dose of gastrin but not by a subthreshold one. When the period of sham feeding was prolonged to 10 min a subthreshold dose of gastrin sufficed to enhance the response. This is in accordance with the results of Olbe (1964), who showed that subthreshold doses of crude gastrin preparations potentiated the effect of 10 min of

sham feeding In 1 dog it was possible to compare the responses to 10 min of sham feeding before and after antrectomy It was then found that the secretory response to sham feeding combined with infusion of 0 06 μg of gastrin/kg/h after antrectomy was higher than the response to sham feeding alone before antrectomy However only after antrectomy was the acid of the main stomach diverted from the intestine by a gastric cannula Normally the duodenum displays acid induced inhibitory effects on the sham feeding evoked secretion (Nilsson 1969) The diversion of acid from duodenum probably contributed to the high response to sham feeding during gastrin infusion after antrectomy

Secretion of pepsin An obvious relation existed between the duration of the sham feeding period and the pepsin response Olbe *et al* (1968) and Vagne and Grossman (1969) have reported that suprathreshold amounts of gastrin inhibit vagally activated pepsin secretion This could not be shown in the present investigation The reason for this might be that the doses of gastrin and pentagastrin used in these experiments were too low to produce such an inhibitory effect Comparisons with the doses of the above mentioned reports are difficult as the authors did not use pure preparations of gastrin

The present results indicate that the amount of gastrin needed to inhibit pepsin secretion after sham feeding in antrectomized dogs is probably greater than the quantity of gastrin normally released in nonantrectomized dogs by sham feeding

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Acta physiol scand 1972 85 33—43

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Uptake of ^{3}H-cortisol by Rabbit Polymorphonuclear Leukocytes in vitro

II Effect of Metabolic Inhibitors and of Temperature on Uptake and Washout

By

BENGT SIMONSSON

Received 25 October 1971

Abstract

SIMONSSON B *Uptake of ^{3}H cortisol by rabbit polymorphonuclear leukocytes in vitro II Effect of metabolic inhibitors and of temperature on uptake and washout* Acta physiol scand 1972 *85* 33—43

Rabbit pseudoeosinophilic polymorphonuclear leukocytes *in vitro* have an uptake of ^{3}H cortisol which is partly saturable and partly non saturable The saturable component was much more dependent on temperature during accumulation and washout than the non saturable component The metabolic inhibitors N ethyl maleimide p chloro-mercuribenzene sulfonic acid iodoacetic acid and 2 4-dinitro-phenol and preheating at 67 C significantly decreased the saturable uptake while the non saturable uptake if anything was increased in some experiments Dinitro-phenol slightly accelerated the washout of the saturable component

The characteristics of the saturable ^{3}H-cortisol uptake strengthens the evidence that it corresponds to a specific interaction with a receptor in the rabbit pseudoeosinophilic polymorphonuclear leukocytes since it behaves like the specific hormone uptake in other tissues under similar conditions The non saturable uptake behaves like a non specific uptake

Rabbit PMN s[1] accumulate ^{3}H cortisol *in vitro* (Simonsson 1971) At physiological glucocorticoid concentrations the uptake is partly saturable and partly non saturable The saturable uptake (*i e* the difference in ^{3}H cortisol uptake between samples incubated in the absence or presence of non radioactive cortisol) has structure specificity and is more sensitive to temperature than the non saturable uptake These characteristics of the uptake indicate that it may be related to a specific sensitivity to cortisol of the cells

To strengthen the evidence that the uptake of ^{3}H cortisol by rabbit PMN s represents a specific uptake it would be valuable to find similarities between this

[1] Abbreviations PMN stands for pseudoeosinophilic polymorphonuclear leukocyt CPM is counts per minute Cortisol denotes 11β 17 21 trihydroxy pregn-4 ene 3 20-dione PHMB p hydroxy mercuri benzoate (sodium salt) PCMS p-chloro mercuribenzene sulfonic acid (monosodium salt) NEM N-ethyl maleimide IAA iodoacetic acid (sodium salt) DNP 2 4 dinitro-phenol DTE di thio-erythritol

uptake and the specific uptake of steroid hormones in other tissues Several workers have shown that the interaction of steroid hormones with receptors in target tissues is sensitive to temperature and SH inhibitors (for a review, see Jensen *et al* 1971) The present work describes the effect of metabolic inhibitors and more detailed studies on the effect of temperature on the uptake and the retention of ^{3}H cortisol by rabbit PMN s

Materials and methods

For details see Simonsson 1971

Compounds

(1 2 ^{3}H) cortisol was obtained from NEN Chemicals Dreieichenhain bei Frankfurt/Main Germany Its specific activity was 42—44 Ci/mmole and its radiochemical purity at delivery was 97 % It was used within 3—4 months Non radioactive cortisol was purchased from Sigma St Louis Mo USA

^{14}C Inulin carboxyl with a specific activity of 1 55 mCi/g was also obtained from NEN Chemicals and the non radioactive inulin from Sigma The glycogen (for chemical purposes) came from Nutritional Biochemicals Corporation Cleveland Ohio PHMB PCMS NEM IAA and DTE were from Sigma and DNP from Kebo AB Stockholm Sweden

Animals

Male albino rabbits weighing 2 5—4 kg were used They were kept at 21 C Commercial rabbit pellets (Harald Fors et Co AB Stockholm Sweden) and tap water were provided *ad libitum*

Some of the animals were subjected to adrenalectomy Since the operation however did not produce any clear cut effect on the uptake of ^{3}H cortisol (Simonsson 1971) it was abandoned

Preparation of PMN s

PMN s were obtained according to the procedure of Hirsch (1956) Male albino rabbits were injected intraperitoneally with warm (38 C) pyrogen free 0 9 % (w/v) NaCl containing 0 1 % glycogen. Four h later 110 ml warm 0 9 % NaCl containing 0 5 % (w/v) Mg EDTA was injected With the needle remaining in the peritoneal cavity the abdomen was gently kneaded and then the fluid drained by gravity into a collecting flask Smears showed that 95—99 % of the white cells were PMN s The volume of erythrocytes was about 3 % of the total cell volume in the exudate The rabbits were injected once a week and they were "primed with at least three injections before they produced exudates rich enough in leukocytes to be used in the experiments

Principle of the in vitro method for separating saturable from non saturable ^{3}H cortisol uptake

A saturable component in the cortisol uptake indicates a specific uptake (*cf* Terenius 1966) The cells were incubated with ^{3}H-cortisol (1 ng/ml) at 37 C In some flasks non radioactive cortisol was added in large excess (1000 ng/ml) This addition suppressed the ^{3}H cortisol uptake by the saturable process The difference in ^{3}H-cortisol concentration between cells incubated with and without non radioactive cortisol was taken as a measure of the amount taken up by the saturable process In the following the simple word uptake will be used for the amount taken up as well as for the process of uptake except where misunderstandings could arise.

Measurement of ^{3}H-cortisol uptake

Siliconized glassware was used The incubation and washing solution (Schaumburg and Bojesen 1968) had the following composition 106 mM NaCl 0 5 mM $MgSO_4$ 1 5 mM $CaCl_2$ 2.3 mM K_2HPO_4 5 7 mM KH_2PO_4 and 8 1 mM glucose The pH was adjusted to 7 4 with HCl. After the peritoneal exudate had been filtered through three layers of gauze to remove any clumped cells and fibrin it was centrifuged and washed twice in ice-cold buffer and finally resuspended to a concentration of 1—2 × 10^{7} cells per ml The cells were then stored at 0 C until the experiment started (1—? h) One ml of a solution containing the radioactive steroid with or without non radioactive cortisol was transferred to open centrifuge tubes and

placed in a water bath shaker (at 37 C when not stated otherwise) The incubation was then started by adding 1 ml of the cell suspension to each tube The final concentration of ^{3}H cortisol was 1 ng/ml and of non radioactive cortisol 1000 ng/ml Three or four identical flasks were always run simultaneously The incubation was terminated by placing the cells in an ice water bath (0 C) Then they were spun down at 300×g for 3 5 min In most expts they were resuspended in 2 ml buffer and washed at indicated time and temperature and finally spun down in the cold at 670×g for 5 min To determine the extra cellular volume in the cell pellet ^{14}C-inulin was added immediately before the final centrifugation The pellets were dried overnight at 100 C They were then weighed and the ^{14}C-inulin was hydrolysed with 0 1 N HCl Finally the pellets and aliquots of the final supernatants were digested with Soluene 100 * The radioactivity was analysed by the liquid scintillation technique The extracellular CPM ^{3}H trapped in the cell pellet were subtracted from the total CPM ^{3}H in the pellet The counts were then converted to CPM × mg dry weight $^{-1}$/CPM × µl incubation solution $^{-1}$ At least 85 % of the tissue radioactivity recovered after thin layer chromatography moved with authentic cortisol No other distinct peakes were found The concentration of radioactivity in the incubation solution decreased less than 1 % on incubation with the cells

Since there are inter individual variations in the total saturable and non saturable uptake of ^{3}H-cortisol (Simonsson 1971) individual controls were run at each experiment.

Results

Effect of temperature on uptake of ^{3}H cortisol

The cells were incubated with the hormone at different temperatures for different times and then washed in ice-cold buffer for 30 min Fig 1 shows that the saturable uptake is most rapid at 37° C and that it is maximal within 10 min after 42 min it has decreased a little At 25° C the process seems to be a little slower but the maximum uptake is larger In another experiment (not shown in Fig 1) the saturable uptake at 25° C seemed not to have reached its maximum within 50 min The non saturable uptake seems to be less temperature sensitive the maximum values are about the same at 25° C and 37° C respectively and they are reached already after 5 min Both the non saturable and especially the saturable uptake are slower at 0° C after 17 hs (= 1020 min) the saturable uptake has not reached the 37° C value and it seems not to have come to its maximum The non saturable uptake at 0° C after 17 h seems to exceed the steady state at 37° C

Fig 2 shows the effect of temperature on the saturable ^{3}H-cortisol uptake The uptake at 37° C is maximal already at 10 min, if the temperature is then reduced to 25° C the uptake increases A re increase in temperature to 37° C gives a decrease to the earlier 37° C level and if the cells are again cooled to 25° C the uptake starts a new increase The decrease in uptake which is caused by the higher temperature is therefore not attributable to an irreversible inactivation of the uptake processes Very little happens if the cell suspension after incubation for 10 min at 37° C is placed in an ice water bath (0° C) for another 32 min This indicates that cooling to 0° C is a good way to terminate an incubation

The effect of temperature on the washout of ^{3}H cortisol

The cells were first incubated with ^{3}H cortisol at 37° C for 25 min and then washed in hormone free buffer at different temperatures for different times Fig 3 shows a

* Packard Instrument International S A Zurich Switzerland

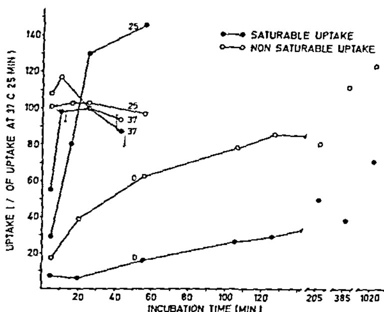


Fig 1 The time-course of ^{3}H-cortisol uptake at different temperatures After the incubation the cells were washed with hormone free buffer at 0 C for 30 min The saturable and the non saturable uptake are calculated as in Methods The uptake is expressed as per cent of the uptake (saturable and non-saturable respectively) after incubation at 37 C for 25 min Vertical bars indicate S E and at these points at least 4 expts were run and each experiment was used as the statistical unit. The other points are from 1 exp each not necessarily in the same animal for any of the curves Three of the 5 expts at 37 C 10 min and 3 of the 4 expts at 37 C 42 min were run with adreneralectomized animals Adrenalectomized animals were also used at 0 C up to 205 min, and at 25 C for 5 and 55 min. The range of the total and of the non saturable uptake in Fig 1 Fig 2 and in Fig 3 usually was less than 15 % of the mean.

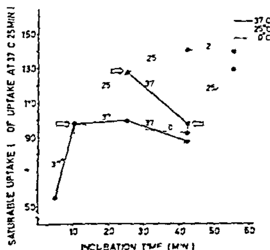


Fig 2 Effect of temperature on the saturable uptake of ^{3}H-cortisol. The experimental conditions were the same as in Fig 1 and the 37 C-curves in Fig 1 and Fig 2 are indentical The arrows indicate for some samples a change in the incubation temperature Vertical bars indicate S E with each experiment used as the statistical unit. Points without vertical bars represent 1 exp Three of the 4 expts. at 37 C 10 min 25 C 15 min were run with adrenalectomized animals

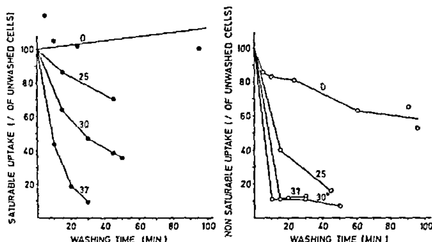


Fig 3 Effect of temperature on the washout of ³H-cortisol The experimental conditions were the same as in Fig 1 except that the cells after the incubation with the hormone were washed at different temperatures for different times Each point is from 1 exp The amount of tritium in the cells is expressed as per cent of the amount of tritium in unwashed cells The points in the 0 C curve are derived from 4 different expts (adrenalectomized animals) and the curve is fitted by sight An adrenalectomized animal was also used at 25 C 15 min.

temperature dependence also in the washout of ³H-cortisol again especially due to the saturable component of the ³H cortisol uptake The non saturable component is easily washed out at 37° C and 30° C while the process is slower at 25° C and slowest at 0° C The washout of the saturable component is also fastest at 37° C and it is decreased by lowering the temperature The retention is very high at 0° C washing for 90 min has no effect In contrast, the non saturable component is reduced by about 35 %

Effect of metabolic inhibitors DTE and pre heating on the uptake of ³H cortiso

In preliminary experiments it was found that metabolic inhibitors decreased the uptake of ³H-cortisol in the PMN's The cells in these experiments were preincubated with the metabolic inhibitor for 15 min at 37 C An aliquot of this cell suspension was then added to an equal part of the ordinary incubation solution with ³H-cortisol and the incubation was run at 37° C for 25 min followed by a wash in standard buffer at 0° C for 30 min Thus during incubation with the hormone the [illegible] concentration was hal ed

To ee if the decrea e in the uptake found in the preliminary experiments [illegible] to an effect on the accumulation or on the washout of the hormone [illegible] treated in two ways (a and b) In a) they were preincubated [illegible] inhibitor for 15 min at 37 C then incubated with hormone and [illegible] as described above and then processed directly without a [illegible] they were incubated with the hormone as in Methods' and [illegible] presence of a metabolic inhibitor at 30° C for 45 min.

TABLE I Effect of metabolic inhibitors di thio erythritol (DTE) and of preincubation at 67 C on the uptake of ^{3}H cortisol by rabbit PMN The cells were first incubated in the presence (experimentals) or absence (controls) of metabolic inhibitor or DTE for 15 min at 37 C The heated group was incubated at 67 C for 15 min One part of the cell suspension (with inhibitor) was then added to one part of the incubation solution containing H cortisol but no inhibitor To half of the samples was added non radioactive cortisol to estimate the non saturable uptake The incubation was then continued at 37 C for another 25 min The cells were then centrifuged in the cold and processed directly ^{3}H cortisol 1 ng/ml cortisol 1000 ng/ml metabolic inhibitor and DTE in half the concentration of preincubation The figures indicate per cent deviation from the controls The metabolic inhibitors were run in 4 different expts and each experiment was used as the statistical unit Italics indicate that the cells were from 1 adrenalectomized animal in all the other expts the cells were pooled from 2 intact animals Significance levels *** = $p < 0\,001$ ** = $p < 0\,01$ * = $p < 0\,05$

Pre incubation conditions	Type of uptake	% deviation from control				Mean ±S E	P
PCMS 10^{-4} M	Total	−28	−4	−33	*−19*	−21±6	*
	Saturable	−100	−100	−100	*−100*	−100±0	***
	Non saturable	+35	+46	+31	*+36*	+37±3	**
IAA $5\times10^{-}$ M	Total	*−31*	*−27*	*−27*	*−34*	−30±2	***
	Saturable	−89	−100	*−70*	−73	−83±7	**
	Non saturable	+20	+10	*+25*	−3	+13±6	
NEM 10^{-4} M	Total	−45	−44	*−31*	−40	−40±3	**
	Saturable	−51	−100	*−100*	−83	−84±12	**
	Non saturable	−42	−13	*+65*	−6	+1±23	
DNP $5\times10^{-}$ M	Total	−18	0	−26	−10	−13 5±6	
	Saturable	−72	−16	−19	−20	−19±1	* *
	Non saturable	−15	+8	−15	0	−5 5±6	
DTE 10^{-4} M	Total	*+6*				+6	
	Saturable	*−1*				−1	
	Non saturable	*+16*				+16	
67 C	Total	+60	*+18*			+39±21	
	Saturable	−73	*−100*			−87±14	*
	Non saturable	+123	*+176*			125±2	***

Table I indicates that *DNP* and the *SH reagents (PCMS NEM and IAA)* decrease the total uptake of ^{3}H cortisol The effect of DNP is however not significant These substances seem to affect the saturable and the non saturable uptake differently the saturable uptake being significantly decreased while the non saturable uptake if anything is increased in some experiments with the SH reagents Table I also shows that DTE an agent reducing S S bridges to SH groups probably has no effect (only 1 exp) Pre heating the cells at 67° C for 15 min inhibits the saturable uptake while the non saturable uptake increases to the double (only 2 expts)

The effect of metabolic inhibitors on the washout of ^{3}H cortisol

It was difficult to reveal any effect of these substances on the washout of ^{3}H-cortisol from the PMN s At 37° C the washout is very rapid (less than 20 % of the saturable uptake is left after 20 min) (Fig 3) and therefore 30° C was chosen The cells were first incubated with ^{3}H cortisol for 25 min at 37° C and then washed with

TABLE II Effect of metabolic inhibitors on the washout of ^{3}H-cortisol from rabbit PMN. The cells were first incubated with ^{3}H cortisol (1 ng/ml) at 37° C for 25 min. To half the samples was added non radioactive cortisol (1000 ng/ml) to estimate the nonsaturable uptake. The samples were then centrifuged in the cold at 300 × g for 3.5 min. Then they were washed by resuspending in 2 ml buffer with the metabolic inhibitor present at 30° C for 45 min. The controls had no inhibitor. The figures indicate per cent deviation from controls. Each treatment was run in 4 different expts and each exp was used as the statistical unit. The cells in each exp were pooled from 2 nonadrenalectomized animals.
Significant levels *** = $p < 0.001$ ** = $p < 0.01$ * = $p < 0.05$

Washing conditions	Type of uptake	% deviation from control				Mean ±S E	P
PCMS 10[illegible] M	Total	−2	−19	−18	−5	−11±4	
	Saturable	+36	−28	−30	−61	−21±20	
	Non saturable	−26	0	+14	+86	+18.5±24	
IAA 5×10[illegible] M	Total	0	+7	−5	+8	+2.5±3	
	Saturable	−45	−6	+3	+8	−10±12	
	Non-saturable	+29	+30	−23	+7	+11±12	
NEM 10[illegible] M	Total	−17	+8	−22	+6	−6±8	
	Saturable	−24	+19	−35	+60	+5±21	
	Non saturable	0	−21	0	−31	−13±8	
DNP 5×10[illegible] M	Total	−28	−11	−25	−14	+19.5±4	*
	Saturable	−45	−28	−38	−22	−33±5	*
	Non saturable	−17	+20	+6	0	+2±8	

[1] The concentration of DNP in this experiment was 10[illegible] M

standard buffer (controls) or either NEM or PCMS present. Samples were analysed for radioactivity after washing for 15, 30 and 45 min. In preliminary experiments only PCMS showed an effect (an acceleration) and it became visible only after 45 min wash. In controls after 45 min only 40 % of the saturable and less than 10 % of the non saturable uptake was left in the cells. Since the experimental error increased when the amount of tritium in the cells decreased, the washing procedure at 30° C was not extended to more than 45 min. The concentrations of the metabolic inhibitors were (except in 1 exp.) those which were previously found to decrease the uptake of ^{3}H cortisol. The scatter in these expts was very large and the results are mainly shown for the sake of completeness. Table IV indicates that DNP probably accelerates the washout of the saturable component and that NEM, IAA and PCMS have not significant effect.

Discussion

In a previous paper (Simonsson 1971) it was suggested that the saturable component in the ^{3}H cortisol uptake by rabbit PMN's was related to a specific sensitivity to cortisol. The non saturable component was suggested not, or at least not entirely, to be related to a specific sensitivity of the cells. The aim of the present work was mainly to strengthen the evidence for these assumptions. A saturable glucocorticoid uptake has been shown in well known target tissues such as thymus (Munck and Brinck Johnsen 1968, Schaumburg and Bojesen 1968), HeLa cells (Melnykovych

and Bishop 1969), hepatoma tissue cells (Baxter and Tomkins 1970) and liver (Beato *et al* 1969) Since there was a structure specificity and in most cases a correlation between the uptake and a biological action of the steroids, the saturable uptake was considered as specific by these authors The structural specificity of the glucocorticoid uptake in the PMN s will be shown in a following paper (Simonsson to be published) A comparison between the findings in accepted target tissues and the present findings shows an extensive parallelism which indicates that the saturable uptake in the PMN s is specific and related to a sensitivity of the cells

Temperature effects

The effect of temperature on accumulation and washout of a hormone is different for specific and non specific uptake The specific uptake is more dependent This is evident from studies on the interaction of glucocorticoids with rat thymus cells and hepatoma tissue cells and the interaction of oestrogens with uterus and diaphragm

Also in the present work the saturable component was more dependent on temperature during accumulation and washout than the non saturable component The rate of the saturable accumulation decreased if temperature was lowered below 37° C This is in accordance with the specific accumulation studied by Schaumburg and Bojesen (1968) and Baxter and Tomkins (1970) In the present work the non saturable accumulation was less dependent on temperature The saturable uptake had a measureable time-course in contrast to the non saturable uptake (except at 0° C) The non specific uptake studied by Munck and Brinck Johnsen (1968) was also very rapid (almost instantaneous) at 37° C while their specific uptake at 37° C was slower and reached maximum within 10 min which is similar to the present findings Differences between the time courses of specific and non specific uptake were also found at 0° C and 17° C by Schaumburg and Bojesen (1968) However while Schaumburg and Bojesen (1968) claimed that the non specific uptake was temperature independent this was clearly not the case in the present experiments

The non saturable uptake after 25 min was about the same at 25° C and 37° C in contrast to the saturable uptake which was higher at 25° C than at 37 C (Fig 2) In the thymus cells the specific glucocorticoid uptake seemed to be maximal at a temperature below 37° C (Munck and Brinck Johnsen 1968) the uptake was highest at 4 C lower at 17° C and lowest at 37° C (Schaumburg and Bojesen 1968) It is not possible to say where the optimal temperature is in the present work, because the incubation at 0 C was probably not run to completion In rat liver nuclei (Beato *et al* 1969) the uptake was higher at 37 C than at 25 C and lowest at 2° C but this might be due to the fact that the incubation was run only for 30 min

The saturable and the non saturable uptake was easier to wash out at 37° C than at a lower temperature The non saturable component ran out a little faster

than the saturable component both at 37° C and 0° C This was the case also for the non specific and specific glucocorticoid uptake respectively at 37° C (thymus cells and hepatoma tissue cells) and at 0° C (thymus cells) There was however no difference in the rate of efflux of specific and non specific bound steroid at 0—4° C in the hepatoma cells The specific and non specific oestrogen tissue inter actions have also many temperature similarities with the saturable and non satur able ^{3}H cortisol uptake respectively in the rabbit PMN (Terenius 1966 and 1968)

Pre heating at 67° C for 15 min almost destroyed the saturable ^{3}H cortisol uptake in the PMN while at the same time the non saturable component rose to the double This is in accordance with the effect of heating on the high and low affinity ^{3}H cortisol binding sites (probably identical with specific and non specific binding sites) in homogenized rat thymus cells (Schaumburg 1970) and the specific cortisol uptake in HeLa cells (Melnykovych and Bishop 1971) Heating also inactivates the corticosteroid binding globulin in the plasma (Daughaday 1959) Ketchel and Garabedian (1963) found an increase with temperature (up to 80° C) in the uptake of ^{14}C cortisol by human leukocytes This uptake therefore was probably non specific

Effect of metabolic inhibitors

The SH inhibitors PCMS NEM and IAA affected the saturable and the non saturable ^{3}H cortisol uptake differently (Table I) The saturable uptake was decreased and it behaved like the specific hormone receptor interactions in other tissues (Beato *et al* 1969 Schaumburg 1970 Melnykovych and Bishop 1971 Baxter and Tomkins 1971 Terenius 1967 Jensen *et al* 1967 Shyamala and Gorski 1969) The non saturable uptake was not decreased (if anything it was increased a little) and this is in accordance with the effect of IAA on the non specific ^{3}H cortisol uptake in HeLa cells (Melnykovych and Bishop 1971) and the effect of SH reagents on the non specific estradiol uptake in diaphragm (Jensen *et al* 1967 Terenius 1967) Melnykovych and Bishop (1971) and Shyamala and Gorski (1969) did not find any inhibiting effect of DNP on hormone receptor binding in contrast to the weak inhibiting effect of DNP on the saturable uptake in the present work On the other hand the DNP effect in the present work is in agreement with the fact that cyanide blocks the specific uptake of 17β-oestradiol in uterus (Terenius 1968) and that Munck and Brinck Johnsen (1968) found that thymocytes required energy (ATP?) for specific ^{3}H cortisol binding Isolated receptor proteins did not need this energy to bind cortisol

SH blocking reagents accelerate the washout of hormone from the target tissue (Terenius 1967 Jensen *et al* 1967 and Beato *et al* 1969) Like in the present work Terenius (1967) could not find any effect of IAA PCMS NEM and IAA could not be shown to have any statistically significant effect on the washout of ^{3}H cortisol (Table II) As mentioned earlier the experimental error increases with increasing washing time because of decreasing dry weight of the cell pellets and

amount of radioactivity This may be the reason why no effect could be demonstrated It is also possible that the lower temperature at which the washout expts were done reduces the effect of the inhibitors PHMB which is much less water soluble and therefore penetrates the cell membrane more rapidly than PCMS (Sutherland *et al* 1967, Rega *et al* 1967, Shapiro *et al* 1970), possibly would have brought about a more clear cut effect on the washout than PCMS DNP accelerated the washout a little

Interpretation

Because of the similarities with the specific interactions between steroids and other target tissues it is probable that the saturable ^{3}H cortisol uptake by the rabbit PMN s corresponds to a specific interaction with a receptor in these cells. The non saturable uptake behaves like a non specific uptake PCMS NEM and IAA react with SH groups on proteins and therefore their effect on the saturable ^{3}H cortisol uptake might be due to an inactivation of a receptor protein in the cells It is known that NEM and PHMB cause a dissociation of cortisol macromolecule complexes in the $100\,000 \times g$ supernatant from thymus cells (Schaumburg 1970) and of a similar oestradiol-complex in the $204\,000 \times g$ supernatant and a nuclear extract from uterus (Jensen *et al* 1967) An increase in washout with temperature may also be due to a binding of the hormone to a receptor protein a binding which becomes firmer with decreasing temperature (compare the cortisol transcortin interaction see Westphal 1971)

On the other hand since PCMS NEM and IAA also are metabolic inhibitors (King *et al* 1966 Tosteson 1966 and Lehninger 1970) their effect on the saturable ^{3}H cortisol uptake may also be due to a blocking of enzymatic processes which furnish energy required to transport the hormone to a receptor in the cell This is supported by the fact that DNP which uncouples oxidative phosphorylation, also decreases the ^{3}H cortisol uptake

The effect of temperature also indicates an energy dependent uptake since the process is more rapid with increasing temperature Gross *et al* (1970) described an exclusion mechanism for cortisol in fibroblasts which has a temperature optimum around 20—25° C and which is inhibited by NEM and incubation at 0° C

As regards retention the evidence for energy dependence is conflicting Against a pump mechanism speaks the fact that there was no leaking out of ^{3}H cortisol at 0° C and for a pump-mechanism speaks the accelerating effect of DNP on the washout

In view of all the evidence it seems however reasonable to suggest that the specific uptake of cortisol in rabbit PMN s consists of 2 components 1) a temperature and possibly energy-dependent accumulation process and 2) a binding process which is firmer at a low temperature than at body temperature Similar conditions seem to be valid for the estrogen uptake and retention in the mouse uterus (Terenius 1967)

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Recurrent Depression from Motor Axon Collaterals of Supraspinal Inhibition in Motoneurones

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Abstract

HULTBORN, H and M UDO *Recurrent depression from motor axon collaterals of supraspinal inhibition in motoneurones* Acta physiol scand 1972 *85* 44—57

The recurrent effects from motor axon collaterals have been examined on inhibitory transmission to motoneurones from the cortico- rubro- and vestibulospinal tracts and a pathway descending in the medial longitudinal fasciculus Disynaptic vestibulospinal IPSPs in knee flexor motoneurones were always considerably decreased by conditioning antidromic volleys No recurrent effects were found on disynaptic IPSPs evoked from the medial longitudinal fasciculus or from the red nucleus in knee extensor motoneurones The transmission of polysynaptic cortical and rubral IPSPs was sometimes recurrently inhibited but more efficiently in flexor motoneurones than in extensor motoneurones Susceptibility to recurrent depression is taken to indicate that the descending IPSPs are mediated by Ia inhibitory interneurones since impulses in motor axon collaterals via Renshaw cells give rise to a selective inhibition of these interneurones Those IPSPs which are unaffected by ventral root stimuli are assumed to be transmitted through other interneurones

Recent investigations have shown that the transmission in the disynaptic Ia inhibitory pathway from muscle spindles to antagonistic motoneurones is effectively depressed by antidromic impulses in motor axons (Hultborn Jankowska and Lindström 1971 a b) This effect is caused by a postsynaptic inhibition of the Ia inhibitory interneurones evoked via *a* motor axon collaterals and Renshaw cells It was furthermore postulated that this recurrent control is selective thus leaving interneurones in other reflex pathways unaffected

In the present study we have tested whether the transmission of IPSPs to motoneurones from various defined descending pathways (a presumed reticulospinal pathway descending in the medial longitudinal fascicle the vestibulospinal the rubrospinal and the corticospinal pathways) also are depressed by preceding volleys in recurrent motor axon collaterals (henceforth referred to as recurrent depression of descending IPSPs The selective recurrent inhibition of Ia inhibitory interneurones may provide a method to differentiate between descending IPSPs mediated by Ia inhibitory interneurones and those mediated by other interneurones The

former IPSPs should thus be susceptive to recurrent inhibition while the latter should be unaffected

It will be shown that there is a regular recurrent depression of vestibulospinal IPSPs suggesting that these to a great extent are mediated via the Ia inhibitory interneurones Cortico- and rubrospinal IPSPs (especially in flexor motoneurones) seem al o in part to be transmitted by these interneurones A preliminar report of some of the findings has been given (Hultborn and Udo 1970)

Methods

The present material was collected from the same experiments which were presented in a preceding paper in which also the experimental procedures were described (Hultborn and Udo 1972) In one additional experiment both the right red nucleus (contralateral to the recorded motoneurones) and the right pyramid (at the Horsley Clarke level of P9) were stimulated In this experiment the sensorimotor cortex was sucked bilaterally and Nembutal anesthesia (25 mg/kg) was given (*cf* Hongo and Jankowska 1967 Hultborn and Udo 1972) After intra cellular recording from motoneurones the microelectrode was regularly withdrawn to a just extracellular position and the field potentials recorded These extracellular field potentials were alwa s negligable as compared with the intracellularly recorded potentials and will therefore not be illustrated in the figures

Abbreviations The following abbreviations are used anterior biceps AB anterior biceps and semimembranosus ABSm deep peroneus (without cutaneous and extensor digitorum brevis branches) DP gastrocnemius and soleus G S posterior biceps and semitendinosus PBSt quadriceps Q cortex cx Deiters nucleus ND medial longitudinal fascicle MLF red nucleus NR ventral root VR postsynaptic potential PSP excitatory postsynaptic potential EPSP inhibitory postsynaptic potential IPSP recurrent inhibitory postsynaptic potential RIPSP flexor reflex afferents FRA (see R M Eccles and Lundberg 1959 Holmqvist Lundberg and Oscarsson 1960 Holmqvist and Lundberg 1961)

Results

Recurrent effects on vestibulospinal IPSPs

A single shock or a short train of pulses in Deiters nucleus usually evoke IPSPs in motoneurones belonging to flexors as well as some hip extensors (Grillner Hongo and Lund 1970 1971) The segmental latency of 1 0—1 4 ms for these IPSPs suggests a disynaptic linkage Such vestibulospinal IPSPs were used as tests which were then conditioned by a preceding stimulation of all ventral roots (L5—S1) besides those evoking a recurrent IPSP in the motoneurone recorded It was always checked that the same conditioning volley depressed Ia IPSPs in the same cell

The present material consists of 17 PBSt and 6 AB motoneurones in which such IPSPs were evoked A typical example of the effect caused by antidromic volleys in motor axons on these disynaptic vestibulospinal IPSPs is given in Fig 1 The depression of the Ia IPSP following an antidromic volley in L5 and L6 ventral roots is illustrated in records A and B The IPSP evoked by a double shock to Deiters nucleus (C) seems to be equally suscept ve to the antidromic conditioning volley (D) as was the Ia IPSP The disynaptic vestibulospinal IPSPs were considerably de creased and sometimes even abolished by preceding antidromic volleys in all the 23 motoneurones tested The depression of the vestibulospinal IPSPs were evoked by conditioning volleys in the same ventral roots which caused the decrease of Ia IPSPs in the same motoneurones (L5 and L6 VRs for AB and PBSt motoneurones *cf*

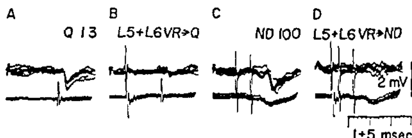


Fig 1 Recurrent depression of a disynaptic vestibulospinal IPSP in a PBSt motoneurone Upper records are intracellular potentials consisting of superimposed traces Lower traces are recorded from L6 dorsal root entry zone In this and the following figures the positivity in intracellular recording and negativity in surface recording are signalled upwards Stimulation strength of peripheral nerves is expressed in multiples of threshold for the lowest threshold fibres Strength of stimulation of the Deiters nucleus is given in μA Voltage (for the intracellular recordings) and time calibration are given in D

Hultborn *et al* 1971 a) The effect from L7 and S1 VRs could however not be properly tested since conditioning stimulation of these roots also evoked recurrent IPSPs (and thus also a conductance increase) in the motoneurones recorded from (*cf* Hultborn *et al* 1971 a)

Recurrent effects on MLF IPSPs

Stimulation of a presumed reticulospinal tract in MLF may evoke IPSPs in knee and ankle extensor motoneurones (Grillner and Lund 1968 Grillner *et al* 1971) These effects were elicited from the same region and at a stimulus strength within the same weak range that gave monosynaptic EPSPs in flexor motoneurones Hence it was suggested that both effects were evoked by the same neuronal system thus giving excitation of flexor and some hip and toe extensor motoneurones and inhibition of other extensor motoneurones (Grillner *et al* 1971)

The possibilities to analyze the effects of ventral root stimulation on the transmission in the Ia inhibitory pathway has proved better in the case of Q motoneurones as compared with G S motoneurones (Hultborn *et al* 1971 a) It was suggested (Hultborn *et al* 1971 a) that the difficulties in the latter case were due to that the most effective roots could not be used for conditioning stimulation since also large RIPSPs were evoked from them In order to show a possible recurrent control of MLF IPSPs the main effort was consequently concentrated on Q motoneurones

In the present material short latency IPSPs were evoked on stimulation of the MLF only in 10 motoneurones (1 G S 9 Q) of 41 motoneurones (7 G S 34 Q) tested In several of the preparations no such IPSPs could be evoked (a train of 3—5 stimuli was always tried) although monosynaptic MLF EPSPs in flexor motoneurones were present

The segmental latencies of the MLF IPSPs studied varied between 1 1—1 5 ms when measured from the initial positive peak of the first effective descending volley (*cf* Grillner and Lund 1968) which is consistent with a disynaptic linkage from

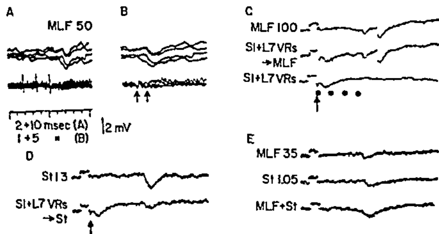


Fig 2 Lack of recurrent depression of a disynaptic MLF IPSP in a Q motoneurone Upper traces in A—B consist of intracellular records and the lower traces are cord surface potentials In C—E all traces are averaged intracellular responses (20 responses) (*cf* in Methods in Hultborn and Udo 1972) The strength of stimulation of the MLF is given in μA In B the first arrow indicates the time of arrival of the initial positive peak of the first effective descending volley and the second arrow shows the onset of the IPSP The points below the lower most trace in C indicate when the stimuli in the MLF were delivered The time of stimulation of L7 + S1 VRs is marked by the arrow in records C and D Voltage and time calibration for A—B are given below A The calibration pulses in C—E have an amplitude of 0 5 mV and a duration of 2 ms

the fastest fibres A monosynaptic connexion from slower fibres seems to be excluded by the finding that a short train (2—3 shocks) always was necessary to evoke the IPSP thus indicating its dependence of a temporal facilitation at an interneuronal level One of the Q motoneurones is illustrated in Fig 2 In order to evoke a di synaptic IPSP from MLF (segmental latency 1 15 ms) 3 shocks had to be used (A and B) Records in C illustrate the consistent finding that the IPSP evoked by stimulation of the MLF were not decreased by preceding volleys in ventral roots Records in D show that a Ia IPSP evoked by stimulation of the PBSt nerve was strongly depressed by a preceding volley in S1 and L7 ventral roots and is thus proving that the lack of recurrent depression of the MLF IPSP was not due to a failure of the recurrent pathway itself Finally the records in E illustrate the common finding that there seems to be no facilitation of the Ia IPSP on conditioning stimu lation of the MLF (Grillner *et al* 1971 Hultborn and Udo 1972) Summarizing our material it can be concluded that no significant recurrent depression of the disynaptic IPSPs from MLF was seen at any occasion

Recurrent effects on rubro pinal IPSPs

The dominating effect on stimulation of the red nucleus is excitation of flexor and inhibition of extensor motoneurones as reported by many investigators (Pompeiano 1957 Sasaki Namikawa and Hashiramoto 1960 Shapovalov and Shapovalova

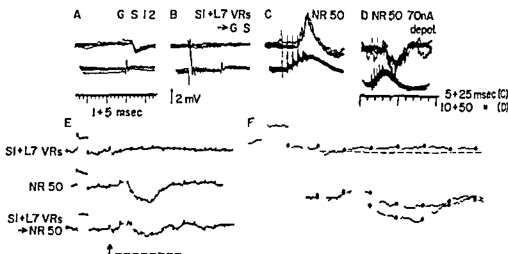


Fig 3 Recurrent depression of rubrospinal IPSPs in a DP motoneurone In A—D the upper traces are intracellular responses and the lower traces cord surface potentials In E—F all traces are averaged intracellular responses The points below the lowermost trace in E indicate when the stimuli in the red nucleus were delivered The time of stimulation of S1 + L7 VRs is marked by the arrow The dashed line below records in E show the part expanded in F where the conditioned and unconditioned rubrospinal IPSPs are superimposed The dashed line together with the uppermost trace in F indicates the horizontal level The strength of stimulation of the red nucleus is given in μA Voltage calibration for the intracellular response in A—D is below B Time calibration for A—B is below A and for C—D below D The calibration pulses in E—F have an amplitude of 1 0 mV and a duration of 8 ms

1965 Kostyuk and Pilyavsky 1967) Later it was pointed out (Hongo Jankowska and Lundberg 1969 a) that there is also frequently evidence of an excitatory action in extensor motoneurones and inhibitory in flexor motoneurones

The effect of conditioning volleys in ventral roots on IPSPs evoked by rubrospinal volleys were tested in totally 50 motoneurones When mixed excitatory and inhibitory effects were evoked from the rubrospinal tract the motoneuronal membrane was depolarized (with current injection through the recording microelectrode) in order to amplify the IPSPs at the expense of the EPSPs In many flexor motoneurones the EPSPs were, however so dominating that even strong depolarization of the membrane did not reveal any rubrospinal IPSPs

In order to exclude that the rubrospinal IPSPs were secondary to firing of motoneurones (and thus mediated via motor axon collaterals and Renshaw cells) it was at several occasions controlled that the rubrospinal volleys did not evoke any discharge in the ventral roots

Fig 3 illustrates a DP motoneurone in which a train of stimuli in the red nucleus evoked an evident EPSP (C) During a depolarizing current (70 nA) the same stimulation of the red nucleus was seen to evoke a pronounced inhibition (D) The short latency IPSP seen in records F and F appeared with the third stimulus and had a segmental latency of 1 3 ms (estimated by the interval between the effective descending volley recorded on the cord surface and the onset of the IPSP)

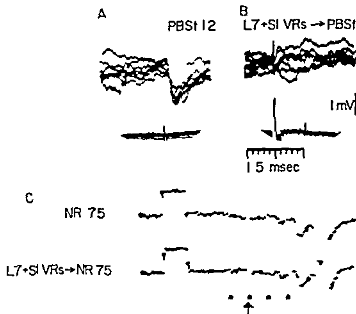


Fig 4 Lack of recurrent depression of a disynaptic rubrospinal IPSP in a Q motoneurone Upper traces in A—B are superimposed intracellular responses and lower traces are cord surface potentials In C both traces consist of averaged intracellular responses The points below the lower trace in C indicate when the stimuli in the red nucleus were delivered The time of stimulation of L7+S1 VRs is marked by the arrow The strength of stimulation of the red nucleus is given in μA Voltage and time calibrations for A—B are given in B The calibration pulses in C have an amplitude of 0 5 mV and a duration of 4 ms

thus suggesting a disynaptic linkage The powerful recurrent control of the transmission in the Ia inhibitory pathway is shown in records A—B (no depolarizing current) In E is illustrated the responses to stimulation of S1 and L7 ventral roots (upper trace) and of the red nucleus (middle trace) and finally to their combined stimulation (lower trace) The effect of the conditioning ventral root volley on the rubrospinal test IPSP is most easily judged from records in F where the unconditioned and conditioned rubrospinal responses are superimposed The disynaptic IPSP was unaffected while the later polysynaptic part was decreased much more than could be explained only by adding the amplitude of the concomitant recurrent facilitation (*cf* upper trace in F) Furthermore the decreased membrane conductance during the recurrent facilitation (Wilson and Burgess 1962 Hultborn *et al* 1971 a) would in itself tend to increase the amplitude of all postsynaptic potentials It can thus be concluded that the recurrent conditioning volley caused a depression of the transmission of the long latency rubrospinal IPSP

The motoneurone illustrated in Fig 4 belongs to the knee extensor Q muscle A conditioning volley in S1 and L7 ventral roots abolished the Ia IPSP evoked from PBSt (A—B) Despite the effective control of the transmission in the Ia inhibitory pathway the disynaptic (segmental latency of 1 2 ms) rubrospinal IPSPs

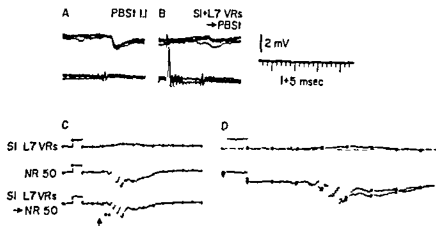


Fig 5 Recurrent depression of a polysynaptic rubrospinal IPSP in a Q motoneurone In A—B the upper traces are intracellular records and lower traces cord surface potentials In C—D all traces consist of averaged intracellular responses The points below the lowermost trace in C indicate when the stimuli to the red nucleus were delivered The time of stimulation of L7+S1 VRs is marked by the arrow In D the unconditioned and conditioned rubrospinal responses are superimposed and expanded The dashed line together with the uppermost trace in D indicates the horizontal level The strength of stimulation of the red nucleus is given in μA Voltage and time calibrations for records A—B in B The calibration pulses in C and D have an amplitude of 1 0 mV and a duration of 8 ms

shown in C were not at all affected by a conditioning volley in the same ventral roots Fig 5 illustrates another Q motoneurone in which disynaptic IPSPs as well as IPSPs of longer latency were evoked by repetitive stimulation of the red nucleus (C—D) As in the Q motoneurone shown in Fig 4 there was no significant recurrent effect on the transmission of the disynaptic IPSPs The late IPSP however seemed to be depressed by the conditioning volley in SI and L7 ventral roots As discussed for the DP motoneurone illustrated in Fig 3 this depression cannot be explained by the minute recurrent facilitatory potential which was evoked in the motoneurone by the conditioning stimulation alone a conclusion which is also supported by the lack of effect on the disynaptic IPSPs

The results are summarized in Table I where the frequency of a recurrent depression of rubrospinal IPSPs in different motor nuclei is presented It appears that

TABLE I *Recurrent depression of rubrospinal IPSPs* In the upper rows are indicated the species of motoneurones investigated as well as the number of cells tested The figures in the lower rows (disynaptic and polysynaptic rubrospinal IPSPs respectively) show for each species of motoneurone separately the number of motoneurones in which a recurrent depression was obtained in relation to the total number of motoneurones tested

motor nuclei	ABSm	Q	CS	FDSt	DP	
number of cells tested	5	30	3	7	5	50
disynaptic depressed/total	—	0/16	—	—	0/1	
polysynaptic depressed/total	3/5	10/25	0/3	4/7	5/5	

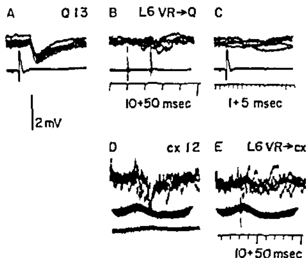


Fig 6 Recurrent depression of a corticospinal IPSP in a PBSt motoneurone Upper traces in A—E are intracellular records Lower traces (middle trace in D) are from L6 dorsal root entry zone Lowermost trace in D is recorded with the microelectrode withdrawn to a just extracellular position and shows the extracellular field potential to the same stimulation which evoked the intracellular response shown by the uppermost trace The dashed line below records in B show the part expanded in C The stimulus strength of cortex is given in mA Voltage calibration for microelectrode recordings Time calibration for A and C below C for B below B and for D—E below E

disynaptic IPSPs in Q motoneurones are never affected by conditioning volleys in ventral roots This was also the case at the single occasion a disynaptic IPSP was tested *in a flexor motoneurone On the contrary the late IPSPs were often depressed* by preceding ventral root volleys both in flexor and extensor motoneurones although more frequently and especially more efficiently in the former group The example of the recurrent depression of a long latency rubrospinal IPSP in the Q motoneurone of Fig 5 in fact constitutes the most evident example obtained while the depression of the late IPSP in the DP motoneurone illustrated in Fig 3 is representative for the findings in flexor motoneurones The depression of late IPSPs in the few ABSm motoneurones collected were either lacking (2/5 Table I) or just liminal (3/5) The recurrent depression of late rubrospinal IPSPs even in flexor motoneurones seems however considerably weaker than that of the Ia IPSPs when tested in the same motoneurones

Recurrent effects on corticospinal IPSPs

The dominant action evoked by volleys in the corticospinal tract consists of a facilitation of flexor and inhibition of extensor motoneurones (for references see Wiesendanger 1969 p 86) However mixed effects are very common especially when the stimulus strength is raised over the minimal range and when current passage through the recording microelectrode was used to analyze a convergence of excitatory and inhibitory actions (*cf* Lundberg and Voorhoeve 1962)

The effects of conditioning volleys in the ventral roots were tested on corticospinal IPSPs in 50 motoneurones At several occasions it was checked that the cortical stimulation did not evoke any discharge in the ventral roots thereby excluding that the cortical IPSPs recorded in motoneurones were secondary to firing of moto

TABLE II. *Recurrent depression of corticospinal IPSPs*. Further description in Table I. Notice that for 9 of the 27 Q motoneurones the pyramid was stimulated instead of the sensorimotor cortex.

motor nuclei	ABSm	Q	PBSt	DP	
number of cells tested	2	27	11	10	= 50
polysynaptic depressed/total	2/2	16/27	10/11	4/10	

neurones. Fig. 6 illustrates a knee flexor PBSt motoneurone in which an unusually profund depression of the corticospinal IPSP was found (D—E). As for all motoneurones it was controlled that the ventral root stimulation used to condition the descending IPSPs effectively depressed the Ia IPSP (A—C). From Table II which summarizes the material on corticospinal IPSPs, it appears that when such IPSPs were evoked in PBSt motoneurones they were almost invariably susceptible to a recurrent control although the depression was often small. There is seemingly a smaller recurrent depression of corticospinal IPSPs in DP motoneurones but most of these motoneurones were gathered in a preparation in which the recurrent effects onto corticospinal IPSPs in PBSt motoneurones, although significant, were less than usual. It may therefore be premature to conclude that there is any significant difference between PBSt and DP motoneurones in this respect. Also if corticospinal IPSPs in Q motoneurones were depressed in 16 motoneurones of 27 these effects were only marginal when compared with those found in PBSt motoneurones. The recurrent depression of the corticospinal IPSPs even in flexor motoneurones was however weak when compared with the efficient recurrent depression of Ia IPSPs.

In 9 of the 27 motoneurones investigated the pyramid was stimulated instead of the sensorimotor cortex. In this experiment also the red nucleus was stimulated (*cf.* Methods). In 4 out of these 9 motoneurones a small recurrent depression could be detected of the pyramidal IPSP. It was of interest to notice that the pyramidal IPSPs occasionally could be depressed in motoneurones in which the rubrospinal IPSP was unaffected and also *vice versa*.

Discussion

Impulses in motor axon collaterals inhibit interneuronal transmission in the disynaptic Ia inhibitory pathway to motoneurones (Hultborn *et al.* 1971 a). A postulate that the IPSPs which are susceptive to recurrent depression are mediated by Ia inhibitory interneurones requires that the recurrent inhibition of interneurones is *confined* to those mediating the Ia reciprocal inhibition. Furthermore, if the lack of recurrent depression of a supraspinal IPSP should exclude a transmission via the Ia inhibitory interneurones it is required that *all* the interneurones mediating reciprocal Ia inhibition are susceptive to recurrent inhibition. We will start to scrutinize these two assumptions.

Although the most striking result in the investigation by Hultborn *et al.* (1971 a) was the efficient recurrent depression of Ia IPSPs in all species of motoneurones,

they also reported that some IPSPs evoked from the ipsilateral FRA occasionally were slightly depressed following ventral root volleys H Hultborn and M Udo (unpublished observations) later found that also IPSPs evoked from the contralateral FRA in flexor motoneurones could be recurrently depressed These findings may appear contradictory to the idea of a selective recurrent inhibition of the interneurones mediating reciprocal Ia inhibition However in this connexion it is important that stimulation of the ipsilateral FRA sometimes facilitates transmission in the Ia inhibitory pathway to extensor motoneurones (Hongo and Lundberg unpublished observations) as well as to flexor motoneurones (Fedina and Hultborn to be published) Similarly it has been established that volleys in the contralateral FRA can evoke a facilitation of transmission in the Ia inhibitory pathway to flexor motoneurones (Bruggencate *et al* 1969) FRA IPSPs transmitted by Ia inhibitory interneurones should of course be susceptible to recurrent inhibition and further investigations (Fedina and Hultborn to be published) support the hypothesis that the FRA IPSPs which are recurrently inhibited are in fact mediated by Ia inhibitory interneurones Similarly when supraspinal stimuli evoked IPSPs susceptive to recurrent depression these stimuli invariably facilitated Ia IPSPs (*cf* Hultborn and Udo 1972) Accordingly recurrent depression of FRA IPSPs or descending IPSPs does not challenge the hypothesis that the recurrent effects on inhibitory pathways to motoneurones are restricted to the interneurones in the reciprocal Ia inhibitory pathway

Nevertheless it has now appeared that also Renshaw cells themselves may receive recurrent inhibition via motor axon collaterals and other Renshaw cells (Ryall 1970 Ryall Piercey and Polosa 1971) It is therefore necessary to consider the possibility that descending IPSPs which might be mediated by Renshaw cells (either secondary to firing of other motoneurones or by direct orthodromic excitation of them) could be depressed by antidromic volleys We often controlled that the supraspinal stimulation did not evoke any discharge in the ventral roots and could therefore exclude the possibility of supraspinal IPSPs mediated by Renshaw cells secondary to excitation of motoneurones but the possibility remains that the descending volleys fire Renshaw cells directly (*i e* not via motoneurones) The existence of such supraspinal direct effects has been postulated (Granit Haase and Rutledge 1960 Haase and van der Meulen 1961 MacLean and Leffman 1967) However disynaptic IPSPs cannot be evoked in motoneurones by this route since there is no evidence for fast descending pathways monosynaptically connected with Renshaw cells (tested with stimulation of the ipsilateral spinal cord in the thorac c region Hultborn and Santini unpublished observations) It is also unlikely that the polysynaptic IPSPs from the corticospinal and rubrospinal tracts are evoked by direct activation of Renshaw cells since the former tract seems to be mainly inhibitory on Renshaw cells (MacLean and Leffman 1967) while no effect was observed from the latter tract (Hongo Jankowska and Lundberg unpublished observations) If nevertheless a minute part of a descending IPSP was to be transmitted by Renshaw cells it is unlikely that even this pa t would be effectively depressed by conditioning

ventral root volleys because the inhibitory interaction between Renshaw cells appears to be very weak (Hultborn, Jankowska, Lindstrom and Roberts 1971).

The problem whether the lack of recurrent depression excludes transmission via Ia inhibitory interneurones is connected with the question whether there exists also a population of Ia inhibitory interneurones which does not receive recurrent inhibition. Hultborn *et al.* (1971 b) discussed this question but found no support for the suggestion of Ia inhibitory interneurones lacking recurrent inhibition. Since the present report deals with the cortico-, rubro- and vestibulospinal systems it is particularly important to recall that the existence of a convergence of excitation from these descending systems on one hand and recurrent inhibition on the other onto Ia inhibitory interneurones has been established (Hultborn and Udo 1972).

Summarizing the above arguments it appears that susceptibility of descending IPSPs to recurrent depression strongly indicates that—and also roughly to which extent—they are mediated by the Ia inhibitory interneurones. On the other hand when the testing conditions are appropriate a lack of recurrent depression virtually excludes that any significant part of a descending IPSP is transmitted via these interneurones.

Our results show that disynaptic vestibulospinal IPSPs in knee flexor and some hip extensor motoneurones are invariably and effectively depressed by conditioning volleys in the same ventral roots which inhibited Ia IPSPs in the same neurones. Hence it is postulated that these descending IPSPs to a great extent are mediated by Ia inhibitory interneurones. Disynaptic MLF IPSPs tested mainly in knee extensor motoneurones were on the contrary never depressed by antidromic conditioning volleys thus indicating that they are mediated via interneurones other than those transmitting the reciprocal Ia inhibition. These differences in susceptibility to recurrent depression shall be compared with the effects from these bulbospinal systems on transmission in the Ia inhibitory pathway—the vestibulospinal tract strongly facilitates Ia IPSPs in knee flexor and some hip extensor motoneurones (Grillner, Hongo and Lund 1966, Grillner and Hongo 1972) while in contrast even weak facilitatory effects from MLF of Ia IPSPs in knee and ankle extensor motoneurones are rare (Grillner *et al.* 1971 and Hultborn and Udo 1972). These findings suggest an interesting disparity regarding the inhibitory connexions from these descending systems. Otherwise the correspondent organization of actions onto α and γ motoneurones is so striking that Grillner *et al.* (1968, 1971) suggested that these two bulbospinal pathways might be functionally coupled and constitute parts of one control system of lumbar motoneurones.

Disynaptic rubrospinal IPSPs were never depressed by preceding ventral root volleys and it is therefore postulated that they are transmitted via other interneurones than those mediating the reciprocal Ia inhibition. This is in accordance with the suggestion that the minimal linkage from the rubrospinal tract to Ia inhibitory interneurones is disynaptic (Hongo *et al.* 1969 b, 1972) although it was not possible to exclude some occasional monosynaptic actions. Recently it was postulated (Baldi[illegible] Bruggencate and Lundberg 1971) that disynaptic rubro-

spinal IPSPs in both extensor and flexor motoneurones can be distributed through the last order interneurones in some private cutaneous reflex pathways

Polysynaptic rubrospinal IPSPs were significantly depressed by preceding ventral root volleys in about half of the motoneurones tested This depression is both more frequent and especially quantiatively more pronounced in flexor than in extensor motoneurones but always less than the depression of Ia IPSPs of corresponding amplitudes Similar results as for the polysynaptic rubrospinal IPSPs were also obtained for corticospinal IPSPs Our conclusion is that the polysynaptic rubro- and corticospinal IPSPs under our experimental conditions to a small but varying part are transmitted via the Ia inhibitory interneurones and more so in case of flexor motoneurones than of extensor motoneurones

Considering that corticospinal and rubrospinal volleys facilitate the reciprocal Ia inhibition to motoneurones (Lundberg and Voorhoeve 1962 Hongo *et al* 1969 b) it is not surprising to find that IPSPs evoked by stimulation of these structures to some degree are secondary to activation of the Ia inhibitory interneurones Since also *eg* Ib and FRA actions receive facilitation from these descending systems (Lundberg and Voorhoeve 1962 Hongo *et al* 1969 b) the largest part of the IPSPs recorded in motoneurones on separate stimulation of them may well be mediated by inter neurones in Ib reflex pathways or by private FRA interneurones (which are not shared with Ia afferents see Lundberg 1970 Fedina and Hultborn to be published) In this connexion it shall be recalled that the effects from the sensorimotor cortex and the red nucleus often parallel the effects from the ipsilateral FRA when recorded in the same motoneurones (Lundberg and Voorhoeve 1962 Hongo *et al* 1969 a) — thus mainly excitation of flexor and inhibition of extensor motoneurones If the descending IPSPs in extensor motoneurones to a large extent were transmitted via private FRA interneurones the smaller susceptibility to recurrent depression of polysynaptic cortico- and rubrospinal IPSPs in extensor than in flexor moto neurones might be explained

Although this discussion has been concerned with the descending effects mediated by interneurones interposed in segmental reflex pathways there is the possibility that also private interneurones—*i e* interneurones activated by the supraspinal systems referred to but unaffected by segmental reflex pathways—would contribute to the descending IPSPs recorded in motoneurones (Vasilenko and Kostyuk 1966 Pilyavsky 1967 Kostyuk and Pilyavsky 1969) However recent investigations (Hongo Jankowska and Lundberg 1972) indicate that such private interneuronal pathways are very unlikely to exist at least in case of the rubrospinal system

Stimulation of some supraspinal centres can probably evoke IPSPs in moto neurones via different interneuronal routes (*cf* above) The contribution from these different pathways will depend on the relative excitability levels of their inter neuronal pools and may be contingent on the state of the preparation In a forth coming paper it will be shown that the IPSPs evoked in motoneurones from the FRA to a varying degree depending on *e g* the anesthesia may be transmitted by the Ia inhibitory interneurones (Fedina and Hultborn to be published)

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The Effect of Acetazolamide upon the Regulation of the Cerebrospinal Fluid pH in the Rat

By

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Abstract

MESSETER K *The effect of acetazolamide upon the regulation of the cerebrospinal fluid pH in the rat* Acta physiol scand 1972 *85* 58—70

The influence of large doses of acetazolamide upon the regulation of the CSF pH in normocapnia and hypercapnia was studied in non nephrectomized and nephrectomized rats In non nephrectomized animals studied over a 24 h period there was a decrease in the CSF HCO_3 concentration of 10 mEq/kg Since the CSF acidosis was far in excess of that usually encountered with a comparable degree of plasma acidosis the results indicate that acetazolamide inhibited a carbonic anhydrase dependent transport of H or HCO_3 between CSF and plasma. However when the fall in plasma [HCO_3] was prevented by means of a bilateral nephrectomy acetazolamide did not affect the CSF pH under normocapnic conditions nor did it retard the accumulation of HCO_3 in the CSF during hypercapnic conditions The results do not support any of the existing hypotheses which try to explain the regulation of the CSF pH in both respiratory and non respiratory acid base disturbances in terms of a common mechanism.

In many tissues which contain carbonic anhydrase the enzyme is associated with the excretion of H or HCO_3 ions (Maren 1967) In the brain the enzyme is located to the choroid plexus and to glial cells (Ashby *et al* 1952 Giacobini 1962) Inhibition of the enzyme affects both the production rate of cerebrospinal fluid (CSF) as well as the distribution ratios for HCO_3 and Cl between plasma and CSF (Davson *et al* 1957 Schwab 1962 Maren 1967) However although these findings and the role played by carbonic anhydrase in other tissues suggest that it might be involved with H or HCO_3 transport also in the brain it is not known if the enzyme is of importance for the regulatory mechanisms which normally maintain the CSF pH within narrow limits in spite of wide fluctuations in the plasma pH (see Mitchell *et al* 1965 Posner *et al* 1965 Fencl *et al* 1966 Fencl 1971)

In man and in most of the animal species studied experimentally the CSF pH is more acid than the arterial or capillary plasma pH in spite of the fact that the CSF is usually electrically positive to plasma (Held *et al* 1964 Fencl 1971) These findings indicate that there are one or several mechanisms which acidify the CSF and maintain an electrochemical potential difference for H and HCO_3 of several mV

between CSF and plasma According to one hypothesis, the acidification occurs by means of an active transport of H or HCO_3 across the blood brain barrier and it has been proposed that purposeful alterations of the rate of ion transport may explain the CSF pH homeostasis (Severinghaus *et al* 1963 Fencl *et al* 1966) However the excess acidity of the CSF may also be explained by an outflux of acid from the brain cells and calculations of electrochemical potential differences for H and HCO_3 between CSF and plasma in sustained acid base changes do not support the hypothesis of an active transport regulation of the CSF pH (Siesjo and Kjallquist 1969 Kjallquist 1970 Sorensen 1971) Instead such calculations suggest that the constancy of the CSF pH may at least partly be explained by the changes in the CSF plasma potential differences (see also Messeter and Siesjö 1971 a)

The hypothesis of an active transport mechanism which normally secretes HCO_3 from CSF to plasma is not compatible with the recent findings of Maren (1971 a) who maintains that carbonic anhydrase-containing cells in the brain primarily secrete HCO_3 into the CSF and he has reported that carbonic anhydrase inhibition reduces the rate of accumulation of HCO_3 in the CSF during acute hypercapnia by about 50 % (Maren *et al* 1971) However although the secretory mechanisms proposed are dissimilar both have been assumed to fulfill homeostatic purposes

The present experiments were undertaken to study the influence of sustained carbonic anhydrase inhibition upon the regulation of the CSF pH in normocapnia and in hypercapnia When acetazolamide was administered to non nephrectomized rats there occurred a marked fall in the plasma HCO_3 concentrations However when the fall in the plasma HCO_3 concentration was prevented by means of a bilateral nephrectomy carbonic anhydrase inhibition did not slow down the compensatory increase in the CSF HCO_3 concentration during hypercapnia In order to facilitate interpretation of the results in terms of the proposed active and passive regulatory mechanisms the CSF plasma potential difference was measured in both nephrectomized and non nephrectomized animals

Methods

General outline of the experiments

In both non nephrectomized and nephrectomized animals acetazolamide (Diamox) was injected i p while the animals were unanesthetized In experiments lasting maximally 12 h a single dose of 50 mg/kg and hr was given whereas in experiments lasting 24 h this dose (i e 600 mg/kg) was repeated after 12 h This large dose was chosen in order to secure complete inhibition of carbonic anhydrase in the long term experiments (Maren 1971 b and personal communication)

In the various groups of animals reported below arterial blood and cisternal CSF were sampled for analyses 4 12 or 24 h respectively after the (first) injection of acetazolamide Since the sampling necessitated anesthesia the animals were anesthetized 30—45 min before the end of the chosen experimental period However in order to make it possible to adjust the arterial P_{CO_2} during anesthesia to that existing in the unanesthetized state control experiments were performed with sampling of arterial blood from indwelling arterial catheters In the anesthetized groups the size of the CSF samples precluded measurements of both the CO_2 content and the CO_2 tension The relationship between the arterial and the CSF CO_2 tensions was therefore determined in a number of animals and this relationship was then used to derive the CSF CO_2 tension from the arterial P_{CO_2} in subsequent experiments

A Non-nephrectomized animals

Group 1 The animals in this group were used to measure the arterial acid base parameters in the unanesthetized state 4, 12 or 24 h, respectively, after the first injection of acetazolamide. In order to allow the animals to recover from the shortlasting anesthesia necessary for the insertion of indwelling tail artery catheters (see Pontén and Siesjö 1967), the operation was performed 2—3 h before the blood sampling.

Group 2 The relation between the CSF and the arterial CO_2 tensions in animals subjected to carbonic anhydrase inhibition was controlled in a small series of experiments. In this and in the following groups the animals were tracheotomized, immobilized and anesthetized as described below and the arterial CO_2 tensions were adjusted to the values observed in group 1.

Group 3 This group consisted of all animals in which arterial blood and CSF were sampled for analyses of CSF-plasma acid base relations. There were 3 groups breathing air and these were analysed 4, 12 and 24 h, respectively, after the first injection of acetazolamide. In addition, a fourth group of animals was exposed to a gas mixture containing 9 % CO_2 and 30 % O_2 starting 45 min after acetazolamide had been injected (Messeter and Siesjö 1971 a). All animals in this group were studied 12 h after the introduction of the CO_2—O_2 gas mixture.

Group 4 In a small series of experiments the electrical D.C. potential was measured between cisternal cerebrospinal fluid and jugular venous blood 4, 12 and 24 h, respectively, after the injection of acetazolamide to air breathing animals, and 12 h after the administration of the drug to animals exposed to carbon dioxide (cf. group 3).

B Nephrectomized animals

In these animals a bilateral nephrectomy was performed through a dorsal skin incision during shortlasting anesthesia with 1 % halothane.

Group 1 The arterial acid base parameters were determined in spontaneously breathing animals (see above). The measurements were performed both in uninjected animals and in animals injected with acetazolamide.

Group 2 The relationship between the CSF and the arterial CO_2 tension was established in rats anesthetized as described below and previously injected with acetazolamide.

Group 3 This group consisted of animals which were injected with a single or with two doses of the drug and which were studied 4 and 24 h, respectively, after the first dose. Since the injection with acetazolamide gave rise to an increase in the arterial CO_2 tension to 50—55 mm Hg, the nephrectomized control groups were exposed to about 5 % CO_2 for similar time periods.

Group 4 In order to allow a comparison with the CSF pH regulation previously observed during sustained hypercapnia in non-nephrectomized rats (Messeter and Siesjö 1971 a), nephrectomized control groups were given about 11 % CO_2 for 3 and 12 h, respectively, while corresponding groups injected with acetazolamide were exposed to about 9 % CO_2. In this way all groups of animals had arterial CO_2 tensions between 80—85 mm Hg. An exposure period of 12 h was chosen since few of the acetazolamide injected animals survived 24 h of hypercapnia.

Group 5 The D.C. potential differences between CSF and jugular venous blood were determined under the experimental conditions described for groups 3 and 4, except for the 3 h hypercapnic group.

Operative and sampling procedures

Between 30 and 45 min before the end of the given observation period the animals were anesthetized with divinyl ether, quickly tracheotomized and artificially ventilated on a Starling type respirator (Braun, Melsungen). After the tracheotomy, anesthesia was maintained on 0.6 % halothane. Muscular relaxation was achieved by intraperitoneal injections of tubocurarine chloride. One femoral artery was cannulated for anaerobic blood sampling and for blood pressure recording. The body temperature was kept close to 37° C by means of intermittent heating. Arterial CO_2 tensions similar to those measured in the unanesthetized state were accomplished either by ventilatory adjustments or by the administration of the same CO_2-containing gas which was delivered to the animals in the box. During operative procedures the arterial pH, P_{CO_2} and P_{O_2} were anaerobically determined by means of microelectrodes with appropriate corrections for the body temperature (Radiometer, Copenhagen and Eschweiler & Co, Kiel). In the majority of experiments and at the end of the experimental period a CSF sample was collected from the cisterna magna. The CSF sample was either analysed for the total CO_2 content by means of a microdiffusion technique (Siesjö 1962) or for the CO_2 tension, using a micro-CO_2 electrode (Eschweiler & Co, Kiel). In some experiments, however, the electrical potential difference was measured between the cisterna magna and an external jugular vein using bridges of 3 M KCl in 2 % agar connected with saturated KCl-calomel electrodes (K 100, Radiometer, Copenhagen). For further details, see Kjällquist (1970), Messeter and Siesjö (1971 b).

TABLE I Arterial acid base parameters measured in unanesthetized and non nephrectomized rats which were given acetazolamide for 4 12 and 24 h respectively The values obtained have been compared to those previously reported for non nephrectomized and uninjected rats (control see Pontén and Siesjo 1967) Number of measurements within parenthesis Means ± S E Non nephrectomized animals

Exposure period	P_{CO} mm Hg	pH	[HCO ₃] mEq/l	BE
Control (21)	38 5 ± 0 5	7 47 ± 0 01	27 3 ± 0 5	+ 4 2
4 hrs (10)	51 2 ± 0 8	7 26 ± 0 01	21 9 ± 0 6	— 4 8 ± 0 8
12 hrs (8)	34 4 ± 0 7	7 29 ± 0 02	15 9 ± 0 4	— 9 2 ± 0 7
24 hrs (10)	35 6 ± 0 9	7 24 ± 0 01	14 6 ± 0 5	—11 6 ± 0 7

Calculations of acid base parameters in blood and CSF

The plasma bicarbonate concentration and base excess values were calculated from the pH and the P_{CO} values using the alignment nomogram of Siggaard Andersen (1963) The CSF bicarbonate concentrations was derived from the measured total CO_2 content by subtracting the physically dissolved CO_2 calculated as the product of the CSF CO_2 tension and the solubility coefficient 0 0314 mmole kg $^{-1}$ mm Hg $^{-1}$ (Siesjo 1962) The experimentally determined relationship between the CO_2 tensions in arterial blood and in the CSF (see below) allowed a calculation of the CSF CO_2 tension from the measured arterial CO_2 tension in all animals injected with acetazolamide *In the nephrectomized but uninjected groups exposed to either* 5 % or 11 % CO the CSF CO_2 tension was derived by adding 4 5 mm Hg to the corresponding arterial value Thus it was assumed that the empirical relationship between the arterial and the CSF CO tension determined in non nephrectomized and non-injected animals exposed to CO_2 (see Messeter and Siesjo 1971 a) was unaffected by the nephrectomy The pH of the CSF was calculated according to the Henderson—Hasselbalch equation using an apparent pk for carbonic acid of 6 125 (Mitchell *et al* 1965)

Results

Non nephrectomized animals

In order to study the effect of carbonic anhydrase inhibition upon the systemic acid base status arterial blood was drawn from indwelling tail artery catheters in spontaneously breathing animals Blood sampling was performed in 3 groups of rats breathing athmospheric air and studied 4 12 and 24 h respectively after the first injection of acetazolamide The arterial acid base parameters obtained under these conditions have been compared to a previous uninjected material (Ponten and Siesjö 1967) in Table I Carbonic anhydrase inhibition led to a transient increase in the CO tension and to a progressive decrease in the bicarbonate concentration The observed decrease in the CO tension at 12 and 24 h only partially compensated for the non respiratory acidosis

Table II gives the mean arterial blood pressure the hemoglobin concentration, the arterial P_O the arterial acid base parameters and the CSF CO content measured in animals previously injected with acetazolamide and anesthetized 30—45 min prior to the end of the observation period The arterial CO tensions obtained under anesthesia were similar to those obtained in the unanesthetized state (*cf* Table I) However the anesthetic procedure gave rise to a slight further decrease of the arterial bicarbonate concentration and of the plasma pH The suggested hemoconcentration together with an observed loss of weight of about 10 % at 24 h

TABLE II. Mean values for the hemoglobin concentration, arterial blood pressure, arterial P_{O_2} and arterial acid-base parameters together with the CSF CO_2 content measured in non-nephrectomized rats injected with acetazolamide and sampled during anesthesia with 0.6% halothane. During anesthesia the arterial P_{CO_2} values were adjusted to values previously determined in animals spontaneously breathing air (cf. Table I). However, since the P_{CO_2} was not measured after 12 h of hypercapnia in the unanesthetized animal, the anesthetized group exposed to 9% CO_2 was given the same ventilation as those breathing air. Number of experiments within parenthesis. Mean ± S.E.

Non-nephrectomized animals

Exposure period in hours	Temp °C	Arterial blood Hb g	MABP mm Hg	P_{O_2} mm Hg	P_{CO_2} mm Hg	pH	$[HCO_3^-]$ mEq/l	BE	CSF CO_2 mmol/kg
4 (4)	37.3 ± 0.2	12.8 ± 0.8	125 ± 9	151 ± [illegible]	51.0 ± 2.6	7.19 ± 0.02	18.1 ± 0.8	−9.8 ± 1.2	22.4 ± 0.9
12 (5)	37.3 ± 0.2	13.7 ± 0.5	110 ± 3	142 ± 7	31.0 ± 1.1	7.25 ± 0.01	11.0 ± [illegible]	−12.0 ± [illegible]	22.3 ± 0.7
21 (5)	37.2 ± 0.1	15.3 ± 0.7	138 ± 3	145 ± 7	32.4 ± 0.5	7.23 ± 0.01	13.3 ± 0.2	−12.7 ± 0.5	19.5 ± 0.5
9% CO_2									
12 (5)	37.4 ± 0.3	14.1 ± 0.2	141 ± 5	164 ± 3	81.5 ± 1.2	6.93 ± 0.01	18.6 ± 0.4	−1?.1 ± 0.1	[illegible]4 ± 1.0

corroborated the known fact that the systemic acidosis was due to bicarbonate diuresis (Goldston 1955, Pitts 1966). The table also shows that there was a progressive decrease in the CSF CO_2 content. However, in the hypercapnic group the CSF CO_2 content was 10 mEq/kg higher than in the group breathing air during the same period.

It has previously been reported that there is a 5–6 mm Hg P_{CO_2} difference between cisternal CSF and arterial blood in animals injected with acetazolamide (Brzezinski *et al.* 1967, Kjällquist *et al.* 1970). This relationship was controlled in the present material. Since there were no significant differences between non-nephrectomized and nephrectomized animals the results were pooled. In nine measurements in a P_{CO_2} range between 27 and 57.0 mm Hg there was a mean P_{CO_2} difference between CSF and arterial blood of 4.6 mm Hg (S.E. ± 0.9). Since the results were close to those obtained in the previous material (Kjällquist *et al.* 1970) the CSF CO_2 tension was calculated for all subsequent acetazolamide experiments by adding 5 mm Hg to the measured arterial CO_2 tension.

In Fig. 3 the acid-base parameters for plasma water and CSF have been illustrated. Knowledge of the CSF/plasma P_{CO_2} relationship allowed calculation of pH and $[HCO_3^-]$ in cisternal CSF. The bicarbonate concentration in arterial blood showed a total decrease of about 12 mEq/kg H_2O of which occurred during the first 4 h. There was a corresponding total decrease in the CSF bicarbonate concentration of about 10 mEq/kg, but no significant change was observed during the first 4 h

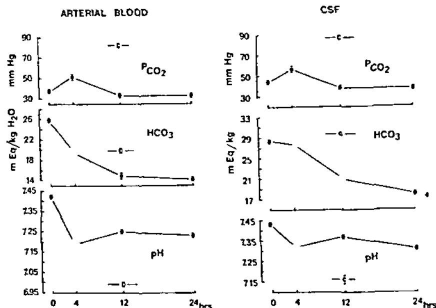


Fig 1 The measured arterial CO_2 tensions and pH values together with the calculated plasma bicarbonate concentrations and the corresponding acid base parameters in the CSF in anesthetized non nephrectomized animals injected with acetazolamide 4 12 and 24 h respectively before analyses The unfilled circles denote values obtained in a group of acetazolamide injected rats exposed to CO_2 (9 %) for 12 h In this and the following figures (2 and 3) the values given at zero time were taken from a previous communication (see Messeter and Siesjo 1971 a) (Means ± S E)

(0 5 < p < 0 6) (*cf* Kjallquist *et al* 1969) Although the fall in the CSF bicarbonate concentration at 12 and 24 h was almost as large as the corresponding decrease in plasma the CSF pH seemed better regulated Thus there was a 0 08 pH difference between CSF and plasma pH at 24 h (see Discussion) The figure also shows that administration of 9 % CO (unfilled circles) completely prevented the fall in the CSF bicarbonate concentration at 12 h Also in hypercapnia the CSF pH seemed better regulated than the arterial pH Thus whereas hypercapnia increased the plasma bicarbonate concentration by 4 mEq/kg plasma water the corresponding increase in the CSF bicarbonate concentration was about 8 mEq/kg H O

Nephrectomi ed animals

Table III gives the arterial acid base parameters measured in the unanesthetized state both in nephrectomized but uninjected animals and in animal nephrectomized injected with acetazolamide As compared to the non nephrectomized uninjected animals (see Table I) the nephrectomized animals showed only a moderate non respiratory acidosis at 24 h In the nephrectomized animals injected w n etazol amide there was a moderate re p ratory acidosis both at 4 and 24 h when compared to the uninjected animals The tab e al o how that there was a comparable respira

TABLE III Arterial acid base parameters measured in unanesthetized nephrectomized rats which were either uninjected or injected with acetazolamide for 4 or 24 h respectively The table illustrates acid base changes in animals exposed to air (4 and 24 h) or to CO_2 containing gas mixtures (3 hrs) In order to achieve arterial CO tensions between 80—85 mm Hg the uninjected group was exposed to 11 % CO_2 as compared to 9 CO_2 in the acetazolamide injected animals Number of experiments within parentheses Means ± S E

Nephrectomized animals

Exposure periods	Uninjected				Injected with acetazolamide			
	P_{CO_2}	pH	[HCO]	BE	P_{CO_2}	pH	[HCO]	BE
4 h	40 5±1 0 (10)	7 44±0 02	24 7±0 8	+2 3±0 4	50 2±1 0 (4)	7 35±0 0	26 8±0 3	+1 4±0 3
24 h	38 6±0 5 (6)	7 36±0 01	21 2±0 6	—3 1±0 7	46 6±1 5 (6)	7 32±0 01	23 4±0 6	—2 1±0 6
	11 % CO				9 % CO_2			
3 h	84 2±0 9 (3)	7 15±0 01	27 6±0 2	—2 7±0 3	84 4±1 6 (11)	7 16±0 01	28 3±0 [illegible]	—2 0±0 5

tory acidosis in the uninjected animals exposed to 11 % CO and in the injected animal exposed to 9 % CO (0 5<p<0 6) It is further shown that 3 h of respiratory acidosis gave rise to only a moderate increase in the plasma bicarbonate concentration in both groups

Since the administration of acetazolamide to the nephrectomized animals gave rise to a respiratory acidosis nephrectomized control groups were obtained by exposing uninjected animals to a 5 % CO gas mixture for 4 and 24 h respectively Table IV shows the mean arterial blood pressure the hemoglobin concentration the arterial P_{O_2} the arterial acid base parameters and the CSF CO contents in nephrectomized animals previously injected with acetazolamide, and in nephrectomized control animals exposed to 5 % CO With the CO containing gas mixture used the control animals had somewhat higher arterial CO tensions than those observed in the animals injected with acetazolamide However these differences will not seriously affect the conclusions Also shown are the corresponding parameters for the acetazolamide injected groups exposed to 9 % CO and for the uninjected control groups exposed to 11 % CO during 3 and 12 h

Fig 2 compares the bicarbonate concentrations and the pH values in arterial blood and in cisternal CSF in the injected and uninjected groups The arterial pH values in the uninjected groups were slightly lower than in the acetazolamide injected groups probably due to the slightly higher CO tension The results demonstrate that the administration of acetazolamide did not lead to any decrease in the CSF bicarbonate concentrations as compared to the uninjected animals In the 24 h animals which had CO tensions rather close to normal the CSF pH calculated therefore did not differ significantly from that of an uninjected group of animals studied under the same anesthesia (Messeter and Siesjo 1971 a) Thus the carbonic anhydrase inhibition did not seem to interfere with the maintenance of a normal pH

TABLE IV Mean values for the body temperature arterial hemoglobin concentration arterial blood pressure arterial P_{O_2} and acid base parameters together with the CSF CO_2 content measured in nephrectomized rats during anesthesia with 0 6 % halothane During anesthesia the arterial P_{CO_2} values were adjusted to the levels previously measured in the unanesthetized state (see Table III) Since the airbreathing groups injected with acetazolamide showed an increase in the arterial CO_2 tensions uninjected control groups were obtained by exposing nephrectomized rats to 5 % CO_2 for 4 and 24 h respectively Number of experiments within parentheses Means ± S E

Nephrectomized animals

Exposure period in hours	Temp C	Arterial blood							CSF
		Hb g	MABP mm Hg	P_{O_2} mm Hg	P_{CO_2} mm Hg	$[HCO_3^-]$ mEq/l	pH	BE	T_{CO_2} m mol/kg
Air + acetazolamide									
4 (4)	36 6±0 1	12 4±0 5	152±9	146±5	51 9±1 4	26 2±0 3	7 32±0 01	—0 2±0 3	35 6±0 3
24 (4)	36 7±0 2	11 6±0 3	120±7	134±11	50 7±1 0	24 1±1 0	7 30±0 01	—2 4±1 0	36 7±1 2
5 % CO_2									
4 (6)	36 9±0 2	13 6±0 5	133±7	155±8	56 6±0 5	25 8±0 5	7 29±0 01	—1 0±0 5	33 4±0 2
24 (4)	36 9±0 1	10 9±0 7	120±14	142±8	56 8±1 0	23 1±0 4	7 24±0 01	—4 6±0 2	34 8±0 6
9 % CO_2 + acetazolamide									
3 (5)	37 3±0 3	13 4±0 2	140±14	152±4	86 0±0 8	27 4±0 6	7 13±0 01	—4 2±0 8	39 8±0 4
12 (5)	37 0±0 1	11 6±0 3	100±3	167±7	87 7±0 6	27 3±0 4	7 15±0 01	—3 8±0 6	43 8±1 0
11 % CO_2									
3 (6)	36 8±0 1	14 9±0 5	140±8	157±5	87 0±1 6	26 7±0 2	7 12±0 01	—5 3±0 4	42.2±0 5
12 (5)	36 5±0 4	14 3±0 1	133±5	157±3	83 3±0 4	26 7±0 3	7 14±0 01	—4 7±0 3	44 2±0 5

under normocapnic conditions provided the plasma bicarbonate was prevented from falling

In order to study the regulation of the CSF pH during hypercapnia nephrectomized rats injected with acetazolamide were exposed to about 9 % CO_2 for 3 and 12 h respectively and the results were compared to those obtained in uninjected but nephrectomized animals exposed to about 11 % CO_2 (Table IV and Fig 3) These procedures resulted in similar CO_2 tensions ($p > 0 4$) and similar plasma bicarbonate concentrations ($p > 0 1$) and pH values ($p > 0 3$) in the injected and uninjected animal The results demonstrate that acetazolamide did not interfere with the accumulation of HCO_3^- in the CSF during the hypercapnia. Thus carbonic anhydrase inhibition was associated with the same degree of CSF pH regulation as in the control animal

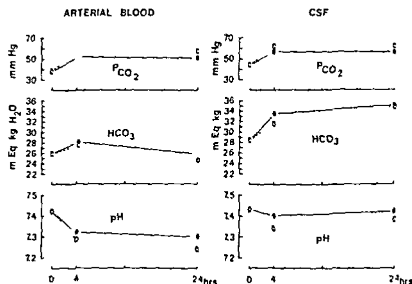


Fig. 2 The acid base parameters in arterial blood and in CSF in nephrectomized control animals given 5% CO_2 (unfilled circles) compared to values obtained in nephrectomized rats subjected to carbonic anhydrase inhibition for 4 or 24 h (filled circles). The left panel gives measured arterial CO_2 tension and pH values together with calculated bicarbonate concentrations. The right panel gives the corresponding CSF values. (Means ±S.E.)

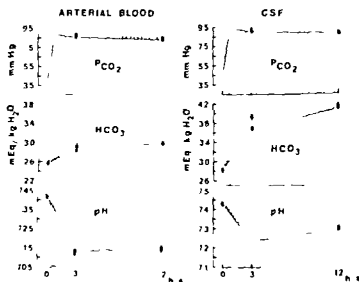


Fig. 3 Mean values [illegible] in arterial blood and CSF from groups of nephrectomized [illegible] (unfilled [illegible]) exposed [illegible] CO_2 for 3 and [illegible] compared to animals [illegible] with [illegible] and exposed [illegible] CO_2 [illegible] periods (filled circles). [illegible] be a [illegible] difference in the accumulation of CSF bicarbonate in the [illegible] pH values [illegible] differ significantly ($0.05 < p < 0.1$). Standard [illegible] given [illegible].

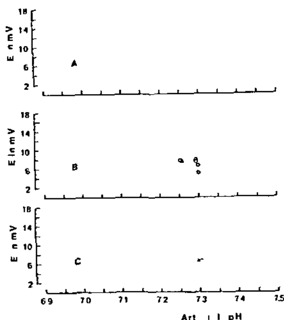


Fig 4 The relation between the arterial pH and the CSF plasma D C potential difference (E) measured in non nephrectomized and nephrectomized animals injected with acetazolamide (A and B respectively) as well as in uninjected nephrectomized animals (C) In non nephrectomized experiments (A) E was determined at 4 h (unfilled circles) 12 h (filled circles) and 24 h (triangles) as well as after 12 h of exposure to 9 % CO_2 (squares) In nephrectomized animals (B) measurements were performed under similar conditions except that no air breathing animals were studied at 12 h In uninjected nephrectomized experiments (C) the relationship was determined in normocapnic and in short lasting hypercapnic conditions (3—4 h) The stippled line indicates the line of regression previously determined in non nephrectomized and uninjected rats exposed to acute and sustained respiratory acidosis (see Messeter and Siesjo 1971 a)

CSF plasma potential differences

Since the flux of H or HCO_3 between CSF and plasma may possibly be influenced by the electrical D C potential (E) between the two compartments the potential was measured between the cisterna magna and the external jugular vein both in non nephrectomized and in nephrectomized animals injected with acetazolamide as well as in uninjected but nephrectomized animals The results showed (see Fig 4) that in all groups of rats the CSF plasma potential seemed to vary with the plasma pH in a manner similar to that previously observed in uninjected and non nephrectomized rats (see Messeter and Siesjo 1971 b and Discussion) However in the non nephrectomized animals injected with acetazolamide (A) there was a suggested further increase in the CSF pla ma potential in tho e groups of rats which were not exposed to CO (4 12 and 24 h)

Discussion

Before discussing the present results it should be pointed out that the evaluation of CSF plasma acid base relations under conditions of an inhibition of blood carbonic anhydrase is hampered by the difficulty of asse ing the true *in vivo* acid base parameters in capillary plasma It is well known that since carbonic anhydrase inhibition retards CO equilibration between blood and ti ue both in the lungs and peripherally *in vivo* measurements of pH P_{CO_2} and $[HCO_3^-]$ in arterial or venous blood samples do not accurately reflect the true or ear capillary values which exist

in vivo (Mithoefer and Davis 1958). This means that quantitative conclusions regarding CSF plasma acid base relations should be drawn with some caution and that comparisons between parameters which depend directly on *e.g.* the plasma pH may not be well justified. Due to these difficulties electrochemical potential differences for H^+ and HCO_3^- between CSF and plasma were not calculated from the present data.

The results obtained in the present experiments on non nephrectomized rats indicate that carbonic anhydrase inhibition affects the regulation of the CSF pH. Thus although a large part of the fall in the CSF [HCO_3^-] may be attributed to the *decrease in the plasma* [HCO_3^-] *the relative decrease in CSF* [HCO_3^-] was larger than what has been reported for chronic non respiratory acidosis and the fall in CSF pH was far in excess of that usually occurring even during marked plasma acidosis (see Fencl *et al* 1966). Since carbonic anhydrase inhibition was associated with an increased CSF plasma potential difference (Fig 4) the results thus fail to support the hypothesis of a "passive" regulation of the CSF pH and they indicate that acetazolamide interferes with a carbonic anhydrase dependent transport of H^+ or HCO_3^- between CSF and plasma.

The tentative conclusions drawn from the results obtained on the non nephrectomized animals could not be corroborated on the nephrectomized animals. Neither did the carbonic anhydrase inhibition affect the CSF [HCO_3^-] in animals breathing air nor did it decrease the rate of accumulation of HCO_3^- in the CSF during hypercapnia. It should be recalled that in the latter situation the CSF [HCO_3^-] constantly was higher than the plasma [HCO_3^-]. Thus provided that only the chemical diffusion gradient determines the flux of HCO_3^- (or H^+) across the blood CSF barrier there should be a continuous loss of HCO_3^- from CSF to plasma in the hypercapnic situations. If we assume that acetazolamide efficiently inhibits the HCO_3^- secretion into the CSF it seems difficult to explain why the acetazolamide injected animals should be able to accumulate HCO_3^- in the CSF as did the uninjected animals when the diffusion gradient for HCO_3^- between CSF and plasma was of similar magnitude. A closer agreement between the present results and those obtained by Maren (1971 *et al*) is obtained if it is assumed that the carbonic anhydrase catalyzed HCO_3^- secretion forms a mechanism for rapid generation of HCO_3^- secretion forms a mechanism for rapid generation of HCO_3^- in the CSF during hypercapnia but that the accumulation of HCO_3^- in the CSF in all situations except the most acute ones when it is determined by other factors.

The results give no unequivocal indication of the presence of a carbonic anhydrase sensitive transport mechanism for H^+ or HCO_3^- or an adequate explanation of the importance of the CSF plasma potential for the distribution of H^+ or HCO_3^- across the blood brain barrier. In the non nephrectomized and injected animals an increased CSF plasma potential difference could not prevent the loss of CSF HCO_3^- (Fig 1). On the other hand in nephrectomized animals the accumulation of HCO_3^- in the CSF during hypercapnia which occurred in spite of carbonic anhydrase inhibition and relatively unchanged plasma HCO_3^- concentration (Fig 3) could be

compatible with a passive flux along an electrochemical gradient The results therefore indicate that different mechanisms may operate in the regulation of the CSF pH in non re piratory and respiratory acid base disturbances

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Reactive Hyperemia in Subcutaneous Adipose Tissue in Man

By

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Abstract

NIELSEN S L and P SEJRSEN *Reactive hyperemia in subcutaneous adipose tissue in man* Acta physiol scand 1972 *85* 71—77

Blood flow in the subcutaneous tissue on the calf was measured with the local Xenon 133 washout method after varying periods of ischemia Reactive hyperemia occurred Maximal flow cumulative flow and excess flow were dependent on the duration of the ischemia The increase in cumulative flow caused by increment in the duration of the ischemic period was characteristic for each individual and was significantly correlated to the resting blood flow This probably indicates adipose tissue blood flow to be governed by local metabolic factors as the tissue only shows a limited autoregulatory response

It is generally assumed in investigations on postischemic hyperemia in limb segments that only cutaneous and muscle tissue participate in the reaction (*e g* Abramson 1967) During rest the blood flow to adipose tissue in a segment of forearm and calf constitutes about 15 % of the total blood flow and a postischemic reaction in this tissue might therefore contribute significantly to the total response of the segment to ischemia

In humans rats rabbits and dogs (Nielsen *et al* 1968 Herd Goodmann and Grose 1968 Lewis and Matthews 1970 Ballard Cobb and Rosell 1971) blood flow in subcutaneous adipose tissue has been found to be associated with the metabolic activity but yet the interrelationship between vascular and metabolic changes in adipose tissue is incompletely clarified (Ross 1971) During shock provoked by bleeding the blood flow in denervated adipose tissue falls rapidly to low values contrary to muscle blood flow and retransfusion causes no reactive hyperemia (Kovach *et al* 1970) However after α adrenergic blockade the response in adipose tissue was converted to a pattern characteristic of tissues showing autoregulation It is therefore likely that adipose tissue deprived of the influence of blood borne catecholamines liberated during shock (for ref see Ballard Cobb and Rosell 1971) would show autoregulation However the flow pressure relationship in vascularly isolated blood

perfused and denervated adipose tissue only showed a poor autoregulatory response (Skinner *et al* 1970)

In the present study the possible occurrence of reactive hyperemia in the subcutaneous tissue in man was investigated and the findings correlated to those of similar studies on skeletal muscle

Material and Methods

5 normal volunteers (medical students and laboratory personnel) were investigated lying dressed at room temperature Blood flow in the subcutaneous tissue on the lateral side of right calf was measured by the local Xenon 133 washout technique (Larsen Lassen and Quaade 1966) after injection of 0 2—0 4 ml isotonic saline containing Xenon 133 (about 1 mCi per ml delivered from AB Atomenergi Studsvik Sweden) 2—4 depots of 0 1 ml were placed along the needle tract by one injection given 60 min before the investigation started

The disappearance rate was registered with a scintillation detector placed 20 cm from the calf pulses being fed into an amplifier/analyzer (Philips PW 4280) and printed every 10 or 60 s from a scaler printer unit without time loss (Meditronic Denmark) The count rates were plotted on semilogarithmic paper versus time after correction for the background activity The washout curves were fitted by two or three straight lines by eye and the half times ($T_{½}$) were read off the curves Blood flow was calculated according to the formula

$$f = \frac{0\,693}{T_{½}} \cdot \lambda \cdot 100\ (\text{ml}/100\ \text{g min}) \qquad (1)$$

where a partition coefficient (λ) for Xenon 133 between tissue and blood of 10 0 was used (Larsen Lassen and Quaade 1966)

The presented flow values during hyperemia is defined as follows 1) Maximum flow is calculated from the steepest initial slope of the Xenon 133 curve (ml/100 g min) 2) Peak flow is maximum flow minus preischemic flow (ml/100 g min) 3) Cumulative flow is the amount of blood flowing through the tissue in the hyperemic period (ml/100 g) It was calculated in 2 ways For the initial washout period cumulative flow is given by maximum flow times duration of increased blood flow the latter being determined as the time until points of intersections of the straight lines fitted to the curve (Fig 1) It was difficult to determine the duration of the hyperemic period occurring after 20 and 30 min of ischemia, because the slope of the second part of the hyperemic phase was only slightly greater than the posthyperemic values Therefore the cumulative blood flow (Cf) in a period chosen to be equal to the duration of ischemia was calculated according to the equation

$$Cf_{0-t} = 2\,3 \cdot \lambda \cdot 100\ (\log N_0 - \log N_t) \qquad (2)$$

where N_0 and N_t are the registered activity at the start and at the end of the period (Lindbjerg 196[illegible] 4 Excess flow is calculated as cumulative flow (Cf) minus the flowdebt which is given by the preischemic flow times duration of ischemia Repayment during hyperemia is calculated as [illegible] flow in per cent of flowdebt

Ischemia was produced by inflating a cuff placed around the thigh to 250 mm Hg In each investigation were performed within [illegible] hours ischemic periods on 2 5 10 20 and 30 min were followed by registration of Xenon 133 washout in 10 10 20 40 and 40 min respectively Only [illegible] of Xenon 133 in isotonic saline was given in a series two series were accomplished [illegible] the 5 persons

The thickness of the [illegible] at the depot site was estimated from the skinfold thickness measured with a Harpenden skinfold caliper gauge

In three persons the [illegible] during the ischemic periods was measured with a constantan-copper [illegible] the skin surface [illegible] the site of injection

Results

The postischemic Xenon 133 washout curves from subcutaneous tissue had a characteristic appearance (Fig 1) In all cases the disappearance rate after 5 10 20 and 30 min of ischemia was increased in comparison to the value measured in the p[illegible]

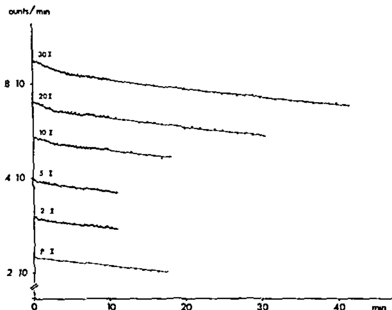


Fig 1 Postischemic washout curves of Xenon 133 locally injected in subcutaneous tissue on the calf in man Time zero indicates release of cuff around thigh Above each curve the duration of ischemia (I) is given the lowest curve being from the preischemic period (PI) The curves are fitted by straight lines

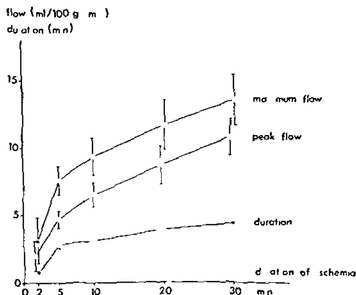


Fig 2 Connection b t een maximum flo peak flo and durat on of the in tial fast wa hout period to duration of ischemia (x ± SE n = 10)

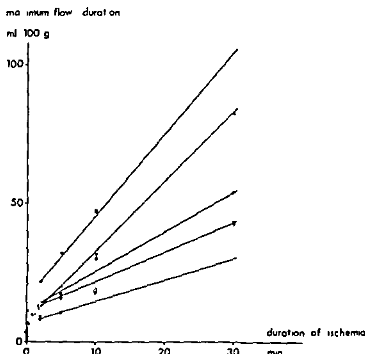


Fig 3 Correlation between cumulative flow in the initial fast washout period and duration of ischemia Each point represents one investigation and the regression line for each individual denoted with different symbols are given

ischemic period After 20 and 30 min of ischemia the count rate decreased 4 4 % (range 1 2—7 6 %) and 5 5 % (range 3 1—9 1 %) respectively within less than 5 min An increase in blood flow after 2 min of ischemia was only distinguishable in 4 cases The hyperemic phase after 2 5 and 10 min of ischemia could be described by one straight line while two were necessary to describe the hyperemic phase after 20 and 30 min of ischemia Paired comparison was made between the values of blood flow calculated from the straight lines of the preischemic and the post hyperemic phase The differences were not significant ($p > 0.40$)

The maximum flow and peak flow corresponding to the fast part of the washout curves showed a linear increase in the duration of the ischemia above 2 min as did the duration of the fast part (Fig 2) The graphical resolution for small and short lasting increments in adipose tissue blood flow after 2 min of ischemia was uncertain After 30 min of ischemia the maximum flow was about 4 times the preischemic blood flow There was however rather great differences in the reactive hyperemia in adipose tissue in the different subjects whereas the responses obtained in the two series performed on the same individual were very similar

The cumulative blood flow in the initial fast washout period after deflating the cuff is shown in Fig [illegible] The values were found to be linearly correlated with the duration of the ischemia [illegible] slope of the regression line characteristic for each experi

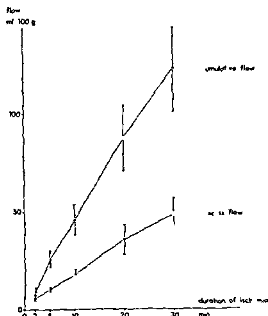


Fig 4 Dependence of cumulative flow (equation 2) and excess flow on duration of ischemia (x ± SE n = 10)

mental subject was significantly correlated to the preischemic flow ($r = 0.89$ $p < 0.01$) but showed no correlation to skinfold thickness

In some investigations a short delay of 20—30 s was seen in the increase of blood flow after 20—30 min of ischemia The 10 s counting period and the small fractional tracer washout made it impossible to quantify the delay in ischemic response as reflected in the washout curves The temperature measured twice in 3 subjects on the skin surface decreased less than 1 5° C during arterial occlusion for 30 min

The cumulative flow (Cf) and excess flow for various durations of ischemia are presented in Fig 4 The linear relationship between these measures of reactive hyperemia and duration of ischemia is obvious Repayment during hyperemia was approximately 80 % after 5 10 20 and 30 min of ischemia on an average 85 % (range 34—159 %) 83 % (range 40—268 %) 82 % (range 22—146 %) and 79 % (range 37—181 %) respectively

Discussion

Redistribution of Xenon 133 by diffusion during the ischemic period from the subcutaneous tissue to tissues with a faster washout than adipose tissue must be considered as a possible basis of the initial faster washout The intercompartmental transfer of inert gas from subcutaneous to cutaneous tissue has been evaluated from the accumulation of Krypton 85 in the cutaneous tissue registered by the β radiation over the skin surface after subcutaneous injection on calf in man (Sejrsen 1967 1969) The exchange between the two compartments was small and slow

about 1 per cent was found to exchange between the 2 compartments the maximum level in vasoconstricted skin being reached in 1 1/2 h With tourniquet the exchange was even slower These results together with the twice as high solubility for Xenon 133 in the subcutaneous tissue lead to the conclusion that during the 30 min of ischemia only about 0 1 per cent of the depot could be exchanged between the 2 compartments Even if a similar exchange might take place to the deeper tissues this is much too little to explain the initial fast washout which after longer periods of ischemia amounted to 5—6 per cent of the registered activity Therefore the increased washout rate must to a major degree represent increase in subcutaneous adipose tissue blood flow as response to ischemia

The reactive hyperemia demonstrated to occur in adipose tissue with the local Xenon 133 washout method exhibits some peculiarities in comparison to the same phenomenon in skeletal muscle (*e g* Lindbjerg 1966) The peak flow after ischemia is small in adipose tissue but it increases continuously with prolongation of the ischemic period In skeletal muscle the peak flow reaches maximum after 5 min of ischemia where blood flow usually is 8—10 times the resting blood flow The smaller capillary density in adipose tissue as compared to skeletal muscle (Gersh and Still 1945) might be responsible for this minor variability in adipose tissue blood flow The blood flow in subcutaneous tissue shows great variation between subjects (Girolamo *et al* 1971) However during standardized conditions the blood flow within a subject is rather constant and physiological vasodilating stimuli can only increase the blood flow 2—4 times Even during dilatation of the vessels in adipose tissue provoked by acethylcholine only a 2—4 fold increase in blood flow was seen (Lewis and Matthews 1970)

The cumulative and excess blood flow in both tissues increases linearly with prolongation of the ischemia but in adipose tissue the increase was significantly correlated to the resting blood flow This might indicate that the postischemic and resting blood flow in adipose tissue is governed by the same regulating factors From the present study it is not feasible to make comparisons to the extensively discussed vasodilating mechanism in skeletal muscle (*e g* Rodbard 1971) Although the basic need in muscle and adipose tissue are essentially the same specific metabolic and vascular reactions in adipose tissue might influence the response to ischemia It is important to note that the flow pressure relationship in subcutaneous tissue in dogs showed a limited autoregulatory response (Skinner *et al* 1970) but postischemic hyperemia was dependent on duration of ischemia (Nielsen and Secher 1970) A similar trend was observed in subcutaneous tissue in rabbits (Nielsen unpublished observations) and is in agreement with the present results The biphasic response seen after 20 and 30 min of ischemia on the washout curves may be more than descriptive but the explanation for this remains uncertain

Regarding skeletal muscle a delay in reaching peak flow has been described (Dahn 1965 Lindbjerg 1966) Comparison with the delay seen in adipose tissue is as mentioned hardly possible because of the difficulty in quantifying the delay For the subcutaneous tissue [illegible] during the ischemia could theoretically be responsible

for a slight delay but the fall in skin temperature was probably too small to cause any vasoconstriction

The reactive hyperemia occurring in adipose tissue can influence the evaluation of postischemic responses in limb segments In the forearm and the calf a 2—4 times postischemic increase in the blood flow of the adipose tissue constituting some 15 % of the limb segment could account for 5—10 % of the total postischemic response The clinical significance of this finding remains to be evaluated

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Acta physiol scand 1972 85 78—90

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Cerebrospinal Fluid Clearance of Choline and Some Other Amines

By

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Abstract

AQUILONIUS S M and B WINBLADH *Cerebrospinal fluid clearance of choline and some other amines* Acta physiol scand 1972 *85* 78—90

The elimination from CSF of the quaternary ammonium compounds choline methylatropine and decamethonium and the tertiary amine atropine has been investigated by ventriculo-cisternal perfusion in anesthetized dogs and rabbits The elimination of the amines from the perfusate was partly concentration dependent Further in the rabbit experiments they decreased the elimination of each other In the dog 63 per cent of the infused choline was eliminated between the lateral ventricle and cisterna magna at an infusate concentration of $5 \cdot 10^{-6}$ M and a rate of 150 μl/min The corresponding figure for the rabbit was 78 per cent at 30 μl/min Brain drug concentrations after the experiments revealed that diffusion into the brain contributed to the elimination only to a minor extent Results were compared to *in vitro* studies on choroid plexa. A correlation was found between the rate of removal from perfusate for the quaternary drugs and their uptake characteristics *in vitro* The conclusion was drawn that the investigated amines are eliminated from CSF partly by a carrier mediated process One important site of this process is probably the choroid plexus
)

Several types of polar substances including some endogenous products and drugs are eliminated from cerebrospinal fluid (CSF) by specific pathways besides diffusion and CSF flow A physiological importance of these pathways seems likely since for instance the monoamine transmitter metabolites homovanillic acid and 5 hydroxyindoleacetic acid are removed from CSF mainly by active transport within the ventricles (Ashcroft Dow and Moir 1968) Consequently the concentration of 5 hydroxyindoleacetic acid is higher in ventricular than in cisternal CSF (Guldberg Ashcroft and Crawford 1966 Andersson 1968) A similar ventriculo-cisternal concentration quotient also exist for the quaternary amine choline (Bowers 1967 Aquilonius Schubert and Sundwall 1970 Further after intraventricular injection the other quaternary amines N methylnicotinamide (NMN) decamethonium and hexamethonium leave the CSF more rapidly than inulin indicating another transport than passive filtration NMN also depresses the clearance of the other amines indicating a

• A preliminary report was presented at the XXX Scandinavian Pharmacological Meeting Copenhagen 1971 (Aquilonius and Winbladh 1971)

carrier mediated transport (Schanker *et al* 1962) Also the elimination of choline from a ventriculo aqueductal perfusate has been shown to decrease somewhat after *i.v.* injection of the bisquaternary amine hemicholinium (Gardiner and Domer 1968)

The choroid plexa have been claimed to be an important site for these transport systems because of their histological character of excretory organs their abundance in all the cerebral ventricles and their ability to accumulate certain substances *in vitro* by means of active transport

The actively transported substances include the organic acids (Cserr and van Dyke 1971) and amines (Tochino and Schanker 1965 Takemori and Stenwick 1966 Eriksson and Winbladh 1971) mentioned above

Lumbar CSF choline concentration has been investigated in an attempt to evaluate central cholinergic activity in man (Aquilonius *et al* 1971) This implies that the lumbar choline concentration reflects that in the ventricles For this reason we found it necessary to evaluate the modes of CSF choline clearance This was done with ventriculo-cisternal perfusion in dogs and rabbits The experiments were designed to elucidate the following questions

I Is the clearance dependent on the concentration of the drug in the perfusate?

II Does addition of a second drug to the perfusate affect the clearance?

III Is there any correlation between the *in vivo* transport rate and the uptake kinetic constants for choroid plexus *in vitro* for a certain drug?

Methods

12 adult beagles of either sex (10—15 kg) were anesthetized with Halothane 2—3 % nitrous oxide 50 % and oxygen 50 % The animals were intubated and allowed to respirate freely The head was fixed in a stereotaxic frame (David Kopf Instrument Inc) and a lateral ventricle cannlutated as described elsewhere (Albanus *et al* 1969) The ventricular cannula was connected to a constant rate infusion pump giving 150 μl/min. Infusion pressure was checked with a Statham transducer (P 23 BC) connected to a Grass polygraph (Grass Instruments Inc.) A short pointed needle introduced into the cisterna magna and connected to a polyethylene tubing served as perfusate drainage In the first 4 expts the opening in the tubing was rised and lowered to obtain a constant outflow approximately equal to the inflow In the rest of the experiments a roller pump drained CSF with a constant rate of 130 μl/min

In the rabbit experiments 30 adult chinchilla rabbits weighing 2 5—3 5 kg were used The experiments were performed essentially as the dog experiments but introduction of the ventricular cannula was made in the coordinates aB according to the rabbit brain atlas of Monnier and Gangloff (1961) The depth to the ventricle was determined by pressure recording during the insertion as described by Goodrich *et al* (1969) for the rat The perfusion rate was 30 μl/min and the ventriculo-cisternal perfusion route was checked by perfusion with trypan blue at the end of several experiments In the 16 last experiments a roller pump drain of 30 μl/min was used In most e pe iments femo l blood pressure was reco ded via a pol eth l n atheter connected to a Statham tran duc (P 23 AC) connected to the polygraph Gr s In truments Inc)

R spiration was recorded w th a Statl am transducer (PT 5 A) connected to the r tube Rectal temperature of bot dogs a d rabbits was kept constant at 38 5 C b ating the animals with electric pad

The perfusate con t d of a b ffer sim lar to CSF[1] with the radioact e a d c t drugs added Inulin is cleared o t o the entricular system almost exclu el by CS ow and

[1] NaCl 7 2 g/l $CaCl_2$ 0 0 g l KCl 0 224 g/l MgCl 0 082 g/l $NaHCO_3$ 2 1 g l NaH_2PO_4 2 H O 0 0/8 g/l and Na HPO_3 H O 0 045 g/l

thus is a good marker for this flow (Heisey Held and Lappenheimer 1962). Therefore ^{14}C inulin was used as a marker for CSF flow while the drugs were ^{3}H labelled. Effluents were pooled in 5 min samples. In order to establish equilibrium each perfusate drug concentration was allowed to run for at least 50 min. Switching between perfusates with different concentrations was accomplished in less than 60 s, special care taken to avoid changes in perfusion speed.

Labelled substances used: choline ^{3}H labelled in a methyl group (15.8 Ci/mmole), methylatropine generally ^{3}H labelled (430 Ci/mole) synthesized from ^{3}H atropine (430 Ci/mole) as described elsewhere (Winbladh 1972), decamethonium tritiated in a methyl group (379 Ci/mole) and inulin carboxyl ^{14}C (3.54 mCi/g, molecular weight 5000—5500). Choline, atropine and decamethonium were purchased from the Radiochemical Centre, Amersham, England and inulin from NEN Corp., Massachusetts, USA. The radioactivity in the perfusate was assayed by liquid scintillation counting with a 3 channel spectrometer (Cpm 2000, Peckman Instruments Inc.). Quench corrections and corrections for channel "cross talk" was done with the internal standard procedure. 50—200 μl of the effluent was mixed with 10 ml of scintillation solvent.[2]

To estimate the penetration of the drugs into the brain substance, gradients were obtained from 7 dogs after the perfusions by pushing a sharpened tube (⌀ 10 mm) from the ventricular side towards the cerebral cortex. The obtained tissue cylinder was then cut transversely in 2—3 mm thick slices and analyzed for radioactivity content. Determination of activity in plasma and brain tissues was made as stated above after oxygen combustion (Eriksson and Winbladh 1971) or acidid ethanol extraction (Albanus *et al.* 1968). When determining total brain concentrations the choroid plexa were dissected out and the whole brain homogenized. 3 samples from each homogenate were analyzed for their radioactivity content as above. Search for metabolites of choline, methylatropine and decamethonium in the perfusates was performed with high voltage electrophoresis as described by Heilbronn and Carlsson (1960) with the acetate buffer modified by addition of pyridine (pH 4.6).

The *in vitro* experiments were performed on choroid plexa from one dog and 8 rabbits as described elsewhere (Eriksson and Winbladh 1971). In short the rabbits were sacrificed by air embolus and the telencephalic choroid plexa were rapidly dissected out. Each choroid plexus was divided into two parts and incubated in Krebs Henseleit buffer with the tritiated drugs added and gassed with 93.5 % O_2 and 6.5 % CO_2. The tissue buffer ratios for the drugs were determined after 30 or 10 min of incubation. Assuming a linear uptake speed the kinetic constants[3] for the uptake were estimated by computer analysis.

Results

General

No backward leakage along the ventricular cannula could be observed either in the dogs or the rabbits. At autopsy no macroscopic changes in the ventricular system were observed except a few ventricular wall petechiae on the perfused side. After trypan blue infusion the colour was spread in all ventricles but not on the brain surface.

No general symptoms which could be ascribed to the perfusions were seen in either dogs or rabbits. Respiration and heart rate did not change significantly when starting the perfusion or increasing the drug concentration in the perfusate. There was sometimes a small increase in blood pressure when starting the perfusions. The perfusion pressure usually showed only minor variations. Experiments in which the

² PPO 5 [illegible] g, POPOP [illegible] g and [illegible] 104 g in 1000 ml of dioxane or Omnifluor (NEN Corp. USA) 4 g in [illegible] 100 and 200 ml [illegible] luene.

³ The kinetic [illegible] along with the constants in the Michaelis Menten equation: V_m [illegible] uptake, K_m = drug concentration at which the uptake is $\frac{V_{max}}{2}$, K_p = the drug [illegible] uptake in presence of an inhibitor is $\frac{V_{max}}{2}$, K_i = inhibitor [illegible]

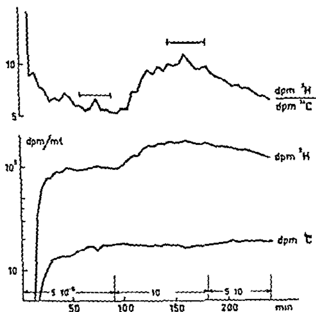


Fig 1

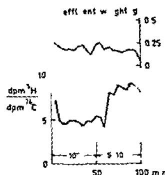


Fig 2

Fig 1 Ventriculo-cisternal perfusion in a dog at different total concentrations of choline but constant ³H-choline and ¹⁴C inulin concentration in the infusate Abscissa gives time in minutes from start of infusion Ordinates show effluent radioactivity (lower curves) and radioactivity ratio (upper curve) Lines above curves marks calculation plateaus

Fig 2 Ventriculo-cisternal perfusion in a rabbit at different total concentrations of choline but constant ³H choline and ¹⁴C-inulin concentrations in the infusate

pressure rose over 15 cm water due to impaired outflow were discontinued 7 rabbits died for unknown reason 25 to 50 min after the change of infusate This occurred after all substances except NMN

Scanning of the high voltage electropherograms of perfusate with methylatropine and decamethonium revealed only one peak indistinguishable from the peak of the infused drug On the scans of the choline perfusates an additional peak was found close to the starting point The radioactivity in this spot represented less than ten per cent of the choline peak as estimated by elution and liquid scintillation counting

In the dog perfusions a plateau in effluent ³H and ¹⁴C activity was reached after an average 60 min (25—100) The corresponding figure for the rabbit perfusions was 20 min (5—45) Fig 1 shows a typical curve when effluent activities are plotted versus time To compensate for the minor variations in inulin concentration i e dilution by CSF the ratio between dpm³H and dpm¹⁴C the radioactivity ratio has been used for calculation of clearance (Fig 1) The statistical analysis of differences between the radioactivity ratios on the different plateaus has been performed with a t test according to Ostle (1954)

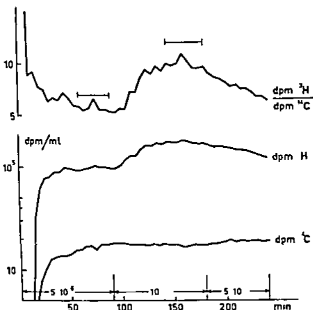


Fig 1

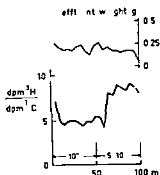


Fig 2

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TABLE I Percentual clearence of drug from the perfusate at different inflow concentrations $\left(1 - \frac{\text{effluent radioactivity ratio}}{\text{infusate radioactivity ratio}}\right) \times 100$

Inflow concentration M	*Dogs mean ± SD*	*Rabbits mean ± SD*
Choline		
5 10	63±11 (n = 4)	8±6 (n = 6)
10	45±15 (n = 3)	49±9 (n = 3)
Methylatropine		
5 10 *	32 36	
10 *	49	
5 10	32 38	66±17 (n = 5)*
10 *	43	
10 *		37±12 (n = 3)*
Decamethonium		
5 10		36±13 (n = 3)
10		5 7±9 0 (n = 3)
Atropine		
5 10		56±14 (n = 4)
10		37±6 (n = 2)

49≠43 ($p < 0.01$) n = number of experiments
* Two rabbit experiments without concentration dependence excluded

The quotient between the radioactivity ratios in the effluent and in the infusate gives the fraction recovered in the effluent. One minus this figure gives the part removed by diffusion and active transport here termed clearance

Effect of increasing perfusate drug concentration

Choline

3 dog and 4 rabbit perfusions were performed and all gave similar results when inflow concentration was raised from 5×10^{-6} M to 10^{-4} M the effluent radioactivity ratio increased in relation to the inflow ($p < 0.01$ in all experiments) indicating a decreased clearance during the passage In the dog experiments and in one rabbit experiment the perfusion period with 10^{-4} M was followed by a perfusion with 5×10^{-6} M Then the effluent radioactivity ratio decreased again ($p < 0.01$) A typical dog experiment is shown in Fig 1 and a rabbit experiment in Fig 2 Clearance values calculated from the mean plateau values are given in Table I The influence of changing the perfusion rate between 15 µl/min and 60 µl/min was investigated in two separate rabbit experiments One of these is shown in Fig 3 Clearance seems inversely related to perfusion rate If the clearance is extrapolated to a perfusion rate of zero a clearance in the order of 90—100 per cent is obtained (Fig 3 inset) Also in one dog experiment clearance was shown to increase when perfusion rate decreased

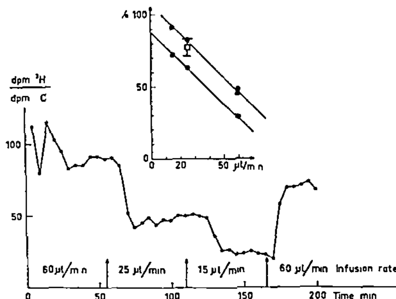


Fig 3 Influence of perfusion rate on radioactivity ratio in a choline perfusion in a rabbit. Inset perfusion rate in relation to clearance in two rabbit perfusions □ mean ± SD of 6 choline perfusions at 30 µl/min

Methylatropine

3 dog perfusions with different concentration ranges were performed In the first two no change in effluent ^3H activity and radioactivity ratio was found when changing inflow concentration from 5×10^{-8} M to 5×10^{-6} M In the third experiment there was a small but statistically significant ($p < 0.01$) decrease in clearance from the perfusate when increasing the concentration from 10^{-6} M to 5×10 M The 7 rabbit experiments did not give consistent results in 3 experiments a clearcut decrease in perfusate clearance was obtained when increasing the infusate concentration ($p < 0.01$) In 2 of these 3 expts concentrations was again lowered and clearance then increased to the initial level In 2 expts only 5 and 6 samples were obtained after the change in perfusate concentration before the animals died but an increase in effluent radioactivity ratio was seen though no plateau was reached However in two apparently satisfactory experiments no significant effect of the change in inflow concentration was seen The results of the methylatropine experiments are summarized in Table I

Decamethonium and atropine

In 3 rabbit expts clearance dec ed when perfusate decamethonium con tration was increased from 5×10^{-6} M to 10^{-4} M (Table I) In 2 of these expts infusate concentration was lowered to 5×10^{-6} M Then clearance returned to approximately

TABLE II Change in clearance of one drug on addition of a second drug [1]

Drug 5 10^{4} M	Added drugs 10^{4} M			
	Methylatropine	Choline	N methyl nicotinamide	Decamethonium
Choline	5 6 4 0		5 0 2 8	28 24
Decamethonium			Not significant (n = 1)	
Atropine		10 6 ?		

[1] Calculated as per cent clearance without — per cent clearance with second drug added Decrease significant on $p < 0\ 01$ level

the same value as during the first perfusion period 2 expts with atropine gave similar results but the difference in clearance between the high and low drug con centration was smaller than that for decamethonium (Table I)

Effect of adding a second drug to the perfusate

The addition of methylatropine in a concentration of 10^{5} M to an infusate with choline 5×10^{6} M did not affect the choline clearance in the 2 dog expts performed Similarly in the dog addition of choline 10^{4} M in 1 expt and 5×10^{5} M in 2 expts had no influence on the clearance of methylatropine in 5×10^{6} M and 5×10^{8} M respectively On the other hand in the rabbit choline clearance from a 5×10^{6} M infusate decreased on addition of 10^{4} M methylatropine NMN or decamethonium ($p < 0\ 01$) in all expts) The effect of methylatropine and NMN was considerably smaller than that of decamethonium NMN 10^{4} M did not significantly influence decamethonium clearance in the rabbit in the single experiment performed Further choline 10^{4} M decreased the clearance of atropine 5×10^{6} M from the perfusate The results of the interaction experiments in rabbits are summarized in Table II

Brain concentrations

Total brain amount of radioactivity in relation to infused amount is given in Table III for both dogs and rabbits In the dog only the slices a and b most close to the lateral ventricle contained significant quantities of ^{3}H activity after perfusion for

TABLE III Total brain content of radioactivity in per cent of infused amount Mean ± SD

Drug	Dog	Rabbit
Choline	3 5 ± 1 5 (n = 3)	19 ± 2 (n = 3)
Methylatropine	0 7 ± 0 3 (n = 3)	9 4 ± 3 5 (n = 2)
Decamethonium	—	7 3 (n = 1)
Atropine	—	5 7 ± 2 (n = 3)

n = number of animals

TABLE IV Brain hemisphere H rad oactivity gradients after dog perfusions

Slice	Per cent of infusate H radioactivity mean ± SD	
	Choline n = 4	Methylatropine n = 3
a[1]	31±7	32±10
b	1 6±0 8	4 3±2 1
c	not significant	not significant
d	not significant	not significant

n = number of animals
[1] close to the ventricle

4—5 h (Table IV) No significant ^{14}C activity was found even in slice a In the dog the ratio between choroid plexus and infusate radioactivity was between 20 and 30 after the choline perfusions while the ratio after the methylatropine experiments was only 2 6 and 0 8 on the perfused side In the rabbit no such large difference between choline and methylatropine was obtained Table V

In vitro studies on choroid plexa

The influence of addition of 3 per cent halothane to the gassing mixture was studied on the uptake of methylatropine in four pieces of choroid plexa. The addition of halothane decreased the uptake approximately 30% as compared to controls Table VI

TABLE V Ratio between choroid plexus and infusate rad oactivity $\frac{\text{dpm H/g tissue}}{\text{dpm H/ml infusate}}$

Concent ation of drug in last per fusion period (M)	Dog		Rabb t	
	Perfused s de	Not perfu sed s de	Perfused side	Not perfu sed side
Choline				
10 *	20 5 26 6	4 0 3 2		
5 10	25 1 29 2	12 3 3 1	16 4 29 7	2 2 2 9
5 10 +NMN 10 *	8 9	0 6	?? 1	0 7
Methylatropine				
5 10	2 6	1 3	29 3 9 1	_9 0 2
10 *	0 8	0 2		
Decamethonium				
5 10			68 3	11 9
5 10 +NMN 10			23 9	9 4
Atropine				
5 10			1 4	
5 10 +Choline 10			1 5	—0 2

Min mally 50 minutes

TABLE VI Tissue medium ratios for methylatropine in rabbit choroid plexus after 40 min in $5 \cdot 10^{-4}$ M medium conc

	Gassing	T/M	Mean	SE
A	O_2—CO_2	21.2 16.2 18.1 18.1	18.4	1.0
B	O_2—CO_2 + Halothane 3 %	15.1 11.9 13.1 11.9	13.0	0.8

[a] Significantly lower than A ($p < 0.01$)

The tissue medium ratios for choline and methylatropine in the dog choroid plexus were $7 \pm$ SD 2 ($n = 4$) and $13 \pm$ SD 6 ($n = 4$) respectively after 40 min of incubation at 10^{-4} M

Uptake of choline and decamethonium in rabbit choroid plexus was concentration dependent Table VII gives the calculated constants Further NMN seems to interfere with the uptake of choline by means of competitive inhibition Table VII

Disappearance of methylatropine from a lateral ventricle in a conscious dog

A cannula was permanently implanted in a lateral ventricle in one dog (10 kg) as described elsewhere (Albanus *et al* 1969) Several weeks later 0.5 μg (1.5 μCi) of tritiated methylatropine and a trace amount of ^{14}C-inulin was given intracerebroventricularly in 50 μl of buffered saline giving a ventricular concentration of about $7 \times 10^{-}$ M of methylatropine

Thereafter 50 μl of CSF was withdrawn every 15 minutes 90 minutes after the first injection a second injection of 0.5 mg of cold methylatropine was given intraventricularly in the same volume As seen from Fig 4 the disappearance rate of labelled methylatropine from the ventricle in relation to inulin was not decreased

TABLE VII Rabbit choroid plexus uptake characteristics *in vitro*

Drug	Inhibitor mM	$V_{max} \pm$ SE mMoles/min kg	K_m $K_p \pm$ SE mM	K_i mM	Number of experiments
Choline	—	0.43 ± 0.19	0.04 ± 0.01	—	8
	NMN 1.0	0.36 ± 0.14	0.10 ± 0.10	0.72	8
Decamethonium	—	0.014 ± 0.001	0.03 ± 0.01		7
Methylatropine	—	0.26 ± 0.02	0.08 ± 0.02	—	30
Atropine[a]	—	*0.35 ± 0.007*	*0.93 ± 0.12*	—	40

[a] Calculated as $\frac{\text{inhibitor concentration}}{\frac{K_p}{K_m} - 1}$ Values from Eriksson and Winbladh 1971

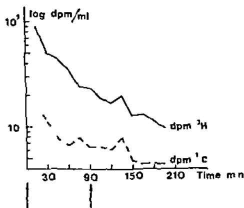


Fig 4 Disappearance of radioactivity from a lateral ventricle in a dog after intraventricular injection of 0 5 µg of ^{3}H methylatropine and a trace amount of ^{14}C inulin at first arrow Second arrow marks intraventricular injection of 0 5 mg of cold methylatropine

Discussion

In the dog choline but not methylatropine was eliminated to a considerable extent from the perfusate by a concentration dependent mechanism The high concentrations of radioactivity in the choroid plexus after the choline compared to the methylatropine perfusions correlate well with this finding However, the *in vitro* experiment with dog choroid plexus showed no tendency to a greater accumulation of choline than methylatropine The interpretation of this result has to be made with caution since it refers to tissue studied at a single medium concentration Further the dog was killed with pentobarbital which might interfere with the uptake (see below) The interaction studies suggest that a low affinity for methylatropine of the hypothetical carrier in the dog choroid plexa might be the reason Anyhow the double injection experiment in the conscious dog and the perfusion experiments make it likely that in the dog a carrier mediated mechanism does not play a major role for the elimination of methylatropine out of CSF For choline the reverse seems true Under our experimental conditions 52—74 per cent of the infused choline was eliminated from the perfusate by diffusion and active transport at an infusate concentration of 5×10^{-6} M This concentration is close to the average ventricular concentration of $4{\cdot}6 \times 10^{-6}$ M found in the conscious dog (Aquilonius Schubert and Sundwall 1970)

As shown in both dog and rabbit experiments clearance decreases when perfusion speed increases This means that clearance as defined in this paper will underestimate drug elimination from CSF in the intact animal Further the anesthesia probably interferes with the transport as demonstrated in the *in vitro* experiments This assumption is supported by the findings that pentobarbital urethan and chloralose decrease choroid plexus uptake of sulphate and iodide *in vitro* and also diminishes the clearance of these substances out of CSF (Lorenzo Hammerstad and Cutler 1968)

In the rabbit choline decamethonium and in five out of seven experiments also

methylatropine were cleared from the perfusate partly by a concentration dependent mechanism. The reason why methylatropine showed no concentration dependence in two experiments is unknown. Possible explanations are unnoticed poor experimental conditions, genetically different transport capacities or intraventricular metabolism of methylatropine to non transported metabolites. Unfortunately neither respiration and blood pressure nor metabolite degradation was investigated in these two experiments.

The clearance consists of two parts: a concentration dependent mechanism and simple diffusion into the tissues along the perfusion routes. With the present technique it is difficult to quantify these two parts exactly. However, an approximation can be made by assuming that diffusion mainly occurs into the brain substance. The diffusion of the used drugs, except atropine, from brain into plasma ought to be very limited because of the brain blood barrier, if they are not metabolized to more lipid soluble compounds. From the data presented in Table III it is seen that between 0.7 and 19 per cent of the infused amount is recovered from brain. The diffusion ought to be faster in the beginning of the experiment when the perfusate brain concentration gradients is greatest. However, the establishment of plateaus in radioactivity ratios during the experiments and the finding that approximately the same plateau could be obtained in the beginning and the end of an experiment contradicts that an important part of the calculated clearance is due to simple diffusion into the brain substance. As seen from Table III choline is recovered from brain to a much higher extent than both methylatropine and decamethonium. It can not be excluded that this depends on an active uptake of choline by the brain substance, since brain slices *in vitro* accumulate choline by means of active transport (Schubert *et al.* 1966).

The interaction experiments in the rabbit make likely that the investigated drugs have one carrier mechanism in common. It is interesting to note that this also seems true for the tertiary amine atropine as has been showed *in vitro* (Eriksson and Winbladh 1971). There are also other correlations between the *in vivo* and *in vitro* findings in the rabbit. The clearance of choline from the perfusate is slightly larger than that of methylatropine and both are considerably larger than decamethonium clearance (Table I). In the *in vitro* studies on choroid plexa V_{max} for choline is somewhat larger than V_{max} for methylatropine, while V_{max} for decamethonium is considerably smaller. K_m seems to be in the same order of magnitude for the three substances (Table VII). Atropine has a V_{max} in the same order of magnitude as choline and methylatropine but a considerably higher K_m (Table VII), and in accordance with this is eliminated from the perfusate to an extent less than the quaternary substances at the low infusate concentration. At the high concentration the difference is smaller (Table I), probably due to a larger diffusion of atropine as mentioned above. NMN is a weak inhibitor of choline transport *in vivo* (Table II). This seems to be the case also *in vitro* (K_i = 0.72 mM), but the standard error of the obtained K_i value is too large to draw any definite conclusions (Table VII).

The above discussed correlation between drug clearance *in vivo* and the drug uptake parameters from studies on isolated choroid plexa supports that these organs

are important for the transport of the substances from CSF to blood[4] Further the ratio in ^{3}H activity between choroid plexus and infusate (Table V) is high after all quaternary drugs found to be eliminated to a considerable extent by a concentration dependent mechanism Consequently after methylatropine in the dog the choroid plexus infusate ratio is low A puzzling observation is that in choline (5×10^{-6} M) perfusion in the dog and decamethonium (5×10^{-6} M) perfusion in the rabbit (Table V) addition of NMN (10^{-4} M) which does not significantly alter drug clearance seems to lower plexus infusate radioactivity ratio

A more detailed calculation of the concentration dependence of the *in vivo* transport appears useless for the following reasons neither the distribution of the perfusate is known in detail nor the drug concentration at the different transport sites Further endogenous choline and possibly other endogenous substances might interfere significantly with the transport as reported for *in vitro* studies on choroid plexus (Tochino and Schanker 1966) Also the anesthesia probably interferes with the transport as discussed earlier

The finding of a higher ventricular than lumbar choline concentration in man makes it likely that there is a removal of choline from CSF between the ventricles and lumbar CSF The ventriculo-lumbar choline concentration gradient in man is in the same order of magnitude as the ventriculocisternal in the dog (Bowers 1967 Aquilonius *et al* 1972) in which probably much less than 40 % of the choline released into the ventricles is recovered in the cisterna magna under normal conditions Thus caution has to be taken when trying to evaluate ventricular choline concentration from lumbar CSF samples in man

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[4] Assuming that the active uptake can be described by an equation analogue to the Michaelis Menten equation for enzyme kinetics the uptake velocity (v) at 5×10^{-6} M drug concentration can be calculated as

$$v = \frac{V_{max}}{\frac{K_m}{5 \times 10^{-6}} + 1}$$

From the data in Table VII the following velocities are obtained choline 0 [illegible] methylatropine 0 1 decamethonium 0 01 and atropine 0 02 mmoles/kg × min per [illegible] of plexus (average weight of one telencephalic plexus 8 mg) $2\,4 \times 10^{-9}$ 10^{-9} 10^{-1} and $?\times 10^{-10}$ moles/min respectively These figures should be compared to the infusate rate of $1\,5 \times 10^{-10}$ moles/min at 5×10^{-6} M infusate drug concentration.

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Effects of Colchicine, Vinblastine and Vincristine on Degeneration Transmitter Release after Sympathetic Denervation Studied in the Conscious Rat

By

DAG LUNDBERG

Received 2 November 1971

Abstract

LUNDBERG D *Effects of colchicine vinblastine and vincristine on degeneration transmitter release after sympathetic denervation studied in the conscious rat* Acta physiol scand 1972 *85* 91—98

The effect of the mitosis inhibitors colchicine vinblastine and vincristine on the time course of the degeneration contraction of the sympathetically innervated periorbital smooth muscle was studied in conscious rats In conformity with earlier findings with colchicine single s.c. doses of vinblastine (2—4 mg/kg) or vincristine (1—2 mg/kg) given at the time of axotomy delayed the start of the degeneration contraction i e the leakage of sympathetic transmitter from the degenerating nerve endings The significance for the delaying effect of the time interval between axotomy and injection of colchicine was studied in some detail The delaying effect declined gradually with increasing time interval between denervation and injection Colchicine given later than 4—6 h after axotomy did not delay In view of this finding and the fact that the mitosis inhibitors used are known to inhibit the intra axonal microtubular transport of different nerve constituents it is proposed that the drugs postpone the degeneration transmitter release by slowing down the rate by which an axotomy information is transported distally along the neuron The axotomy information probably linked to some axotomy induced change in intra axonal structures related to the microtubules seems to be transported at a rate of about 4 mm per hr Local treatment of the decentralized superior cervical ganglion with colchicine or vinblastine induced a degeneration contraction probably due to a complete inhibition of the flow of some vital matter to the nerve endings

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At certain time intervals after axotomy of postganglionic autonomous neurons various events in the nerve terminals are induced Some of these are rather well studied such as leakage of stored transmitter (cholinergic Emmelin and Strömblad 1957 adrenergic Goodall 1951 Euler and Purkhold 1951 Sears and B[illegible] 1960) deterioration of the axonal [illegible] amine pump mechanisms in adrenergic neurons (Malmfors and Sachs 1965 Sm[illegible] 1966) and the appearance of electron microscopic degeneration signs in adrenergic nerve endings (van Orden *et al* 1967) The factors which determine the latency between the axotomy and the onset of the degeneration changes in the nerve terminals are only slightly known Emmelin (1967)

however, has shown that after postganglionic parasympathetic denervation the transmitter leakage starts earlier if the distal part of the degenerating neuron is short than if it is long Reasonably some kind of a signal descends the axon from the cut end and starts the degeneration processes in the nerve endings Mitosis inhibitors such as colchicine are known to interfere with the intra axonal transport of different nerve constituents probably by disrupting neurotubules (for ref see Dahlstrom 1971 a) In previous experiments (Lundberg 1970 d) a single s c injection of colchicine was found to delay the start of the degeneration release after sympathetic denervation in the rat if it was given at the time of axotomy This finding may indicate that the descending axotomy information is related to an intra axonal transport of some kind The present experiments were undertaken in order to study this possibility further

Materials and Methods

Experimental animals

Male Sprague Dawley rats weighing about 250 g were used They were kept in normal day light conditions at around 23 C Commercial rat food pellets (no 210 Anticimex Sollentuna, Sweden) and tap water were provided ad lib

Surgical procedure

Except in 1 expt see Results the right superior cervical ganglion was extirpated (denervation) on the left side the preganglionic trunk was cut (decentralisation) The operations were performed under ether anesthesia

Drugs

Commercial colchicine was used Vinblastine sulphate (Velbe®) and vincristine sulphate (Oncovine®) were kindly donated by Eli Lilly Sweden AB Stockholm The drugs were dissolved in 0 9 % NaCl with 10 I E per ml of testicular hyaluronidase (Hyalas®) just before use and injected s c Hyalas® was kindly donated by Leo AB Helsingborg Sweden Doses refer to the salts

Measurement procedure

The width of the palpebral aperture which is controlled by the tone of the sympathetically innervated periorbital smooth muscle was measured in the conscious rats at a distance using the method described in detail earlier (Lundberg 1969) The palpebral apertures of the two eyes were measured simultaneously about once an hour between 12 and 26—30 h after the denervation The difference in mean width of aperture between the denervated and the decentralized (control) side at every occasion of measurement was plotted against the time after denervation The difference curve (the degeneration contraction curve) obtained was then analysed The time corresponding to 50 % of maximum effect on the ascending part of the curve was used as the time of start of the degeneration contraction (T_{50}) the time of 50 % peak effect on the descending phase was taken as the time of end of the contraction (T_{50d}) The height (the amplitude) of the contraction was the maximum value noted on the denervated side during the degeneration contraction no regard being taken to the decentralized side

Student's t test was used for the analysis of significance

Results

Effect of colchicine given at different times after denervation

A single s c injection of colchicine at 1 mg/kg was given to rats in groups of 4—5 at different times during the interval between the denervation and the expected onset of the degeneration contraction The results are shown in Fig 1 and Table I

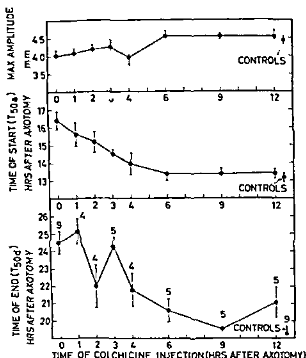


Fig 1 Effect of single s c injections of colchicine (1 mg/kg) given at different time intervals after denervation (axotomy) on the degeneration contraction The maximum amplitude is the peak aperture measured on *the denervated side during the degeneration contraction* (the height) T50 is the time of half maximum development of the contraction T50d is the corresponding time on the descending phase The number of rats of each group is shown in the lowermost curve by the figure placed above the vertical bar The vertical bars show S E M

Fig 1 shows clearly that the start of the degeneration contraction was delayed if colchicine was given within the first 6 h after denervation The delaying effect declined gradually with increasing time interval between denervation and injection Although less distinct a similar trend was visible also with respect to the end of the contraction. Thus colchicine caused a real delay of the degeneration contraction The uppermost curve of the figure shows the maximum amplitude of the contraction. There was a slight tendency to decreased peak amplitudes in the groups given colchicine during the first 6 h after denervation In the 4 h group there were comparatively narrow palpebral apertures on both sides throughout the experiment see Table I The reason for this effect is not clear In the other groups the width of aperture on the decentralized side was not significantly influenced by the treatment

15—20 h after the injection of colchicine the rats became increasingly lethargic ataxic and had decreased muscular tone No rat died (0/41) within 40— 0 h after the injection.

TABLE I Effect of single s c injections of colchicine (1 mg/kg) given at different time intervals after denervation (axotomy) on the degeneration contraction T_{s} is the time of half maximum development of the contraction $T_{s,d}$ is the corresponding time on the descending phase Significance levels are *** = $p < 0.001$ ** = $p < 0.01$ and * = $p < 0.05$

Time of injection h	n	Time of start ($T_{s,s}$) h	Delay of start (T_{s} exp — T_{s} contr) h	Time of end ($T_{s,d}$) h[1]	Height (peak aperture on den side) mm	Aperture on dec side mm
0	9	16.43±0.51 ***	3.23	24.48±0.72 ***	4.03±0.15 *	2.65±0.17
1	4	15.67±0.71 ***	2.75	25.19±0.77 ***	4.10±0.091 *	2.29±0.26
2	4	15.20±0.59 **	2.28	22.01±1.23 **	4.19±0.12	2.46±0.071
3	5	14.50±0.20 **	1.30	24.31±0.50 ***	4.24±0.19	2.89±0.12
4	4	13.97±0.58	1.05	21.82±1.02 *	3.87±0.18 *	2.05±0.13 *
6	5	13.36±0.29	0.16	20.60±0.71	4.51±0.14	3.03±0.18
9	5	13.36±0.25	0.16	19.55±0.45	4.54±0.032	3.03±0.16
12	5	13.40±0.20	0.20	21.13 ±0.19 *	4.51±0.22	3.02±0.19
untreated rats	9	13.20±0.22	—	19.32±0.34	4.48±0.078	2.58±0.12

h after denervation

[1] magnitude of palpebral aperture on the decentralized side when the degeneration contraction had its peak on the denervated side

Effect of vinblastine or vincristine given at the time of denervation

Rats in groups of 4 were given single s.c. doses of vinblastine or vincristine at the time of operation The results are shown in Table II The drugs delayed the degeneration contraction in a similar way as colchicine In the group given 2 mg/kg of vincristine one rat had no visible degeneration contraction and the contractions of the other had distinctly reduced amplitudes This tendency was also seen after vinblastine at 4 mg/kg The toxic symtoms induced by vinblastine or vincristine were similar to those of colchicine 2 out of the 4 rats given 2 mg/kg of vincristine died at about 40 h after the injection

Degeneration contraction after local treatment of the decentralized superior cervical ganglion with colchicine or vinblastine

In connection with bilateral decentralisation the superior cervical ganglions were treated as follows and then left in situ The results are shown in Table III In 4 rats colchicine (100 µg in 5 µl 0.6 % NaCl) was injected under the epineurium of the right ganglion with a Hamilton syringe (701 N) and needle gauge 26 0.9 % NaCl was injected similarly into the left ganglion In all rats there were degeneration contractions on the colchicine treated side but not on the control side The contractions had normal appearances and magnitudes but appeared somewhat later

TABLE II Effect of vinblastine or vincristine on the degeneration contraction The drugs were injected s c at the t me of denervation(axotomy) T_s is the time of half development of the contract on T_d is the corresponding time on the descending phase S gnificance levels as in Table I

Drug and dose	n	Time of start (T_{os}) h	Delay of start (T_{os}exp — T_s contr) h	Time of end (T_{od}) h	Height (peak aperture on den side) mm	Aperture on dec side* mm
Vinblastine 1 mg/kg	4	14 19±0 30	0 74	** 21 33±0 34	4 75±0 13	2 80±0 17
Vinblastine 2 mg/kg	4	14 81±0 60	1 36	* 21 99±1 35	4 43±0 17	2 85±0 12
Vinblastine 4 mg/kg	4	*** 18 68±0 71	5 23	*** 25 58±0 24	** 3 70±0 19	2 38±0 18
Vincristine 1 mg/kg	4	* 14 79±0 40	1 34	* 21 39±0 17	4 71±0 24	2 99±0 27
Vincrist ne 2 mg/kg	3/4*	18 7 15 7 } 18 4 20 7	5 25 2 25 } 4 3 7 25	26 1 24 9 >25 7	3 0 3 4 } 3 1 2 9	2 2 ? 2 } 2 2 2 4
Sal ne	8	13 45±0 23	—	19 59±0 26	4 53±0 11	2 56±0 14

h after denervation
magnitude of palpebral aperture on the decentralized s de when the degenerat on contraction had its peak on the denervated s de
* in 1 out of 4 rats there was no degeneration contract on

TABLE III Degeneration contraction after local treatment of the decentralized superior cervical ganglion with colchicine or vinblastine Colch c ne (100 µg) was injected under the epineurium vinblastine (0 001 M) was administered by soaking the gangl on for 10 min Surgically ganglionectomized rats were run simultaneously and used as controls The colch c ne and vinblast ne exper ments were run on rats bought from two d fferent an mal breeders which may explain the difference between the two control group T_{os} is the t me of half development of the contraction T_d is the corre ponding time on the descend ng phase S gnificance levels as in Table I

Treatment des gn	n	Time of tart (T) h*	Time of nd (T_{od}) h	Height (peak aperture on treat side) mm	Aperture on control s de mm
Colchicine njected into the gangl n	4	16 42±0 44	24 89±0 59	4 45±0 73	? 93±0 34
Surg cal gangl onectomy	5	15 74±0 36	?4 48±0 58	4 25±0 17	45±0 11
Soaking of the gangl on with Vinblastine	4	14 14±0 1	* 22 4?±0 50	4 59±0 52	? 83 0 048
Surg cal gangl onectomy	4	1 73 ‿ 19	19 79±0 44	4 67±0 12	2 85±0 55

h after operation
magnitude of palpebral ap tur — con rol side when the deg neration cont tion had its peak on the treated side

than after axotomy (*cf* the surgical controls) In another group of rats (n=4) vinblastine was used for the local treatment The right ganglion was soaked for 10 min with a cotton pellet impregnated with vinblastine (0 001 M in 0 9% of NaCl with 10 I E per ml of testicular hyaluronidase Hyalas®) The left ganglion was treated similarly with the NaCl Hyalas solution There were degeneration contractions of normal type on the vinblastine treated side in all rats but no reaction on the control side Since vinblastine solutions have low pH values (0 01 M has pH 4 5 see Dahlstrom 1971 a) it was tested in 5 rats whether similar soaking of the ganglion with a biphtalate buffer at pH 4 0 induced degeneration contraction This was not the case in any rat

Discussion

The degeneration phenomenon in the present study reflects the spontaneous release of stored transmitter from the degenerating noradrenergic nerve terminals It is normally very constant with respect of time of start duration and magnitude This property makes it suitable for studies on drugs and factors which influence the time course of degeneration processes in noradrenergic nerves (Lundberg 1970 a) In previous studies it has been shown that bretylium and some other quaternary ammonium compounds delay the degeneration transmitter release most probably by interferencing with degeneration processes at the level of the nerve endings (Lundberg 1970 b and c) However what is the signal for the start of the peripheral processes that initiate the transmitter leakage? Emmelin (1967) has shown that the shorter the distance between the cut end of an excised autonomous neuron and the nerve terminals the earlier the degeneration transmitter release starts Thus the axotomy information is *presumably transported distally along* the neuron The message transported could either be a negative information *i e* the absence of the normally transported nerve constituents or a positive one consisting of something formed at the cut end of the neuron Theoretically both kinds of message could rely on the function of the microtubular intra axonal transport system which has received much attention lately (see Dahlstrom review 1971 a) The mitosis inhibitors colchicine vinblastine and vincristine are known to be bound selectively to a specialized protein in the microtubules of various types of cells including nerve cells (Borisy and Taylor 1967 Bensch *et al* 1969) Local treatment of nerves with *colchicine or vinblastine inhibits the intra axonal flow of different nerve* constituents such as amine storage granules (Dahlstrom 1968) acetylcholine esterase (Kreutzberg 1969) and protein labelled with H^3 leucine (Karlsson and Sjostrand 1969) probably by disrupting the neurotubules

Systemic injections of colchicine vinblastine or vincristine and the time course of the degeneration transmitter release

In a previous study it was shown that colchicine given subcutaneously at the time of axotomy delayed the degeneration contraction of the rat periorbital smooth muscle (Lundberg 1970 d) It was proposed that the delaying effect of colchicine could be due to its ability to disrupt neurotubules thereby slowing down the rate

of the transport of the axotomy information This proposal is probably supported by the findings of the present study the delaying effect of colchicine is shared by the pharmacologically closely related vinca alkaloid and the colchicine effect which is seen only after early administrations of the drug declines gradually with increasing time interval between the denervation and injection The latter fact may mean that the later colchicine is given the longer the process of information tran port has advanced with its normal rate and the less the possibility of the drug to lengthen the total time needed for the transport When the information has reached its goal colchicine treatment is without effect According to this theory the nerve terminals seem to be informed of the axotomy after about 5 h but the release of the transmitter does not start until 8 h later If the axotomy information is sent off immediately at the time of the axotomy and if the distance between the superior cervical ganglion and the eye in the rat is taken to be 20 mm the rate of information transport is 4 mm per hour This rate of transport is similar to that calculated for the intra axonal transport of particulate organelles such as amine storage granules in the rat sciatic nerve *i e* 3—5 mm per hour (Dahlström and Haggendahl 1966 1971) In a recent ultrastructural study (Banks *et al* 1971) it was shown that local treatment with low concentrations of colchicine or vinblastine on constricted rat hypogastric nerves only seemed to affect the transport of microtubules and granular vesicles of the non myelinated axons This finding may indicate that the axotomy information consists of some axotomy induced change either of the negative or the positive kind see above related to these subcellular structures

The mitosis inhibitors used tended to diminish the magnitude of the degeneration contraction The mechanism of this effect is not clear However Keen and Livingston (1970) have recently found that systemic vinblastine (3 mg/kg) reduced the noradrenaline contents of the spleen heart and vas deferens in the rat

Degeneration transmitter release after local treatment of the decentralized superior cervical ganglion with colchicine or vinblastine

Injection of colchicine under the epineurium of the decentralized ganglion or soaking of the ganglion with vinblastine induced spontaneous transmitter release similar to that following surgical ganglionectomy Identical treatment techniques were used by Dahlstrom in fluorescence histochemical studies on rat sciatic nerves (colchicine injection 1963 and rat superior cervical ganglion (vinblastine soaking 1971 b) She found pronounced accumulation of fluorescent material above the area of the colchicine in tion and in the case of the vinblastine treated cervical ganglion there was a clear-cut disappearance of the fluorescent nerve endings of the iris and ptosis which started 18—24 h after the operation indicating degeneration of the sympathetic nerves It is tempting to assume that in the present study the local treatment colchicine or vinblastine caused such a severe inhibition of the microtubular intra axonal transport of some vital matter to the nerve endings that a chemical denervation was induced.

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Effects of Secretin, Cholecystokinin and Caerulein on Gastric Secretion in Response to Sham Feeding in Dogs

By

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Abstract

SJODIN L *Effects of secretin cholecystokinin and caerulein on gastric secretion in response to sham feeding in dogs* Acta physiol scand 1972 85 99—109

Secretin (0 75—3 0 U/kg/h) did not regularly inhibit the acid secretory response to 10 min of sham feeding to a significant degree in 3 dogs with intact antrum Cholecystokinin (0 75—3 0 U/kg/h) and caerulein (0 06—0 30 μg/kg/h) tended to increase acid output after sham feeding in these dogs In antrectomized dogs the response to sham feeding was markedly enhanced by cholecystokinin (0 6—2 4 U/kg/h) and caerulein (0 06 μg/kg/h) Pepsin output after sham feeding was increased by secretin (3 0 U/kg/h) and tended to be reduced by cholecystokinin (3 0 U/kg/h) and caerulein (0 30 μg/kg/h) These findings indicate that gastric acid secretion in response to sham feeding is not inhibited by physiological amounts of secretin and in fact increased by cholecystokinin and caerulein The pepsin output after sham feeding can be enhanced by secretin and possibly depressed by chol cystokinin The physiological significance of these effects on pepsin secretion remains to be determined

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Secretin has been shown to be a potent inhibitor of gastric acid secretion stimulated by gastrin (Greenlee *et al* 1957 Johnson and Grossman 1969) Likewise cholecystokinin and the related decapeptide caerulein in low doses have been found to inhibit gastrin induced acid secretion (Gillespie and Grossman 1964 Stening Johnson and Grossman 1969 Johnson and Grossman 1970) These findings have led some authors to conclude that in the dog secretin and cholecystokinin are important physiological inhibitors of gastric secretion (Johnson and Grossman 1969 1970 Odori and Magee 1970)

Pepsin secretion has been s[illegible]n to be stimulated by secretin (Pratt 1940 [illegible]ka jima Nakamura and Magee [illegible]96[illegible] while cholecystokinin has been found to have more variable effects being [illegible] both to stimulate (Stening and Grossman 1969) and inhibit (Nakajima a[illegible] [illegible] [illegible]970)

The present study was undertaken in order to investigate the effects of the three peptides on gastric acid [illegible] [illegible]sin secretion stimulated by sham feeding

Parts of this investigation [illegible]ere p[illegible]e[illegible]ented at the Nobel Symposium XVI—Fro[illegible]iers in Gastrointestinal Hormone Re[illegible]earch—S[illegible]oc[illegible]holm 1970

Methods

Surgical procedures 8 mongrel dogs weighing 12—26 kg selected for their good appetite were used They were prepared with an esophageal cannula according to the technique described by Olbe (1959) All dogs were also provided with an innervated mucosal septal fundic pouch (Pavlov pouch) and a gastric cannula (inner diameter 2 cm) The gastric cannula was placed in the most dependent portion of the corpus part of the stomach In 3 of the 8 dogs prepared in this manner an initial series of experiments was performed before further preparations were made When this series of experiments (first series) had been completed antrum along with a 2—3 cm strip of corpus mucosa was removed in 2 of the dogs Gastrointestinal continuity was restored by gastroduodenostomy A third dog not included in the initial experiments was also prepared in this way In the remaining 4 dogs the antrectomy was combined with removal of the duodenal bulb In these dogs gastrointestinal continuity was restored by gastrojejunostomy All antrectomies were performed after that the antrum-corpus border had been visualized by the pH paper indicator technique (Andersson 1960 Olbe 1963) The completeness of the antrectomies was checked by histologic examinations In 6 dogs the completeness was confirmed In the 7th dog (dog B) the histologic examination showed that the margin of resection was close to the antrum corpus border indicating a possibility that all antral mucosa had not been removed After each operation the dogs were normally allowed a period of 3 weeks for recovery before the experiments were started

Experimental procedures The dogs were deprived of food for 18—20 h before each experiment The animals were used on alternate days not more than three times a week The gastric cannula was kept open throughout all experiments Basal secretion was registered for 1 h Samples were collected for 15 min or 1 h periods from the pouch and for 1 h periods from the main stomach Volume was read to the nearest 0 1 ml and acid content determined by titration with 0 01 N NaOH with phenolphthalein as indicator Pepsin concentration in hourly samples of juice from the pouch was determined by the hemoglobin substrate method (Bucher Grossman and Ivy 1945) When basal secretion had been recorded an intravenous infusion of 0 15 M NaCl with or without a peptide was started and continued throughout the experiments When given alone secretin and 0 15 M NaCl were infused for 4 h Cholecystokinin caerulein and gastrin were administered for 5 h When secretin was infused concomitantly with another peptide the administration of secretin was also continued for 5 h When 2 hormones were administered simultaneously they were dissolved separately and infused in separate veins All infusions were given at a constant rate of 25—30 ml/h by calibrated peristaltic pumps (Harvard Apparatus Co Dover Mass USA) After 1 h of infusion of 0 15 M NaCl or secretin alone or after 2 h of infusion of the other peptides alone or in combination with secretin the dogs were sham fed for 1—30 min Immediately before the sham feeding procedure the esophageal cannula was opened and a piece of gauze was tied tightly around the esophagus below the cannula in order to prevent food from passing down to the stomach Food was never observed to leave the gastric cannula after sham feeding The dogs were sham fed minced meat or fish

Three main series of experiments were performed

First series 3 dogs with intact antrum were sham fed for 1 and 10 min during concomitant infusion of secretin (0 75—3 0 U/kg/h) cholecystokinin (0 75—3 0 U/kg/h) caerulein (0 06—0 30 μg/kg/h) or in control experiments 0 15 M NaCl Combinations of cholecystokinin and secretin were used in 2 dogs

Second series 3 antrectomized dogs with gastroduodenostomies and 1 with gastrojejunostomy (dog G) were sham fed for 10 min during infusion of 0 15 M NaCl or synthetic human gastrin I (0 06—0 30 μg/kg/h) either alone or combined with secretin (3 0 U/kg/h)

Third series 4 antrectomized dogs with gastrojejunostomies were sham fed for 1—30 min during infusion of 0 15 M NaCl secretin (0 24—2 4 U/kg/h) cholecystokinin (0 24—4 8 U/kg/h) or caerulein (0 012—0 06 μg/kg/h)

Test substances Natural caerulein a gift from Professor V Erspamer Rome Italy

2 preparations of porcine cholecystokinin were used A commercial preparation with an activity of 250 U per mg was used in most experiments It was kindly supplied by Professors E Jorpes and V Mutt of the Gastro-Intestinal Hormone Research Unit Karolinska Institutet Stockholm Sweden In some experiments pure cholecystokinin was used This was a gift from Professors Jorpes and Mutt The dosage of cholecystokinin is given in Ivy dog units

Gastrin (synthetic human gastrin I) was kindly supplied by Dr L V Allan of ICI Pharmaceuticals Division Alderley Park England

Pure porcine secretin was kindly supplied by Professors Jorpes and Mutt The dosage of secretin is given in clinical units

Statistical evaluation of data The results are expressed as the means plus and minus the S E The effect of secretin cholecystokinin and caerulein on sham feeding responses in in

TABLE I Acid responses to sham feeding (SF) in 3 Pavlov pouch dogs with open gastric cannulae during concomitant i v infusion of 0.15 M NaCl or secretin

Dog	Number of expts	Period of SF min	Dose of secretin U/kg/h	Acid output mEq/3 h	Change as of control value	Degree of significance
A	7	10	0	2.29±0.35	—	—
	4	10	0.75	3.00±0.65	+31	p > 0.05
	6	10	1.5	2.32±0.41	+ 1	p > 0.05
	4	10	3.0	0.34±0.14	−86	p < 0.05
B	10	10	0	1.27±0.23	—	—
	3	10	0.75	1.59±0.66	+25	p > 0.05
	3	10	1.5	0.71±0.12	−44	p > 0.05
	4	10	3.0	1.11±0.50	−13	p > 0.05
	4	1	0	0.77±0.15	—	—
	3	1	3.0	0.09±0.05	−88	p < 0.05
C	9	10	0	7.60±0.43	—	—
	3	10	3.0	5.91±1.38	−22	p > 0.05
	7	1	0	2.76±0.66	—	—
	3	1	3.0	3.46±0.43	+25	p > 0.05

dividual dogs was analysed by comparing mean acid and pepsin outputs for 3 h after sham feeding during infusion of each agent with the corresponding response during the control experiments. The t test for unpaired values was used in the statistical analysis (Snedecor and Cochran 1967). In the second series, in addition to the analysis of the secretin effect in individual dogs, its effect on the dogs taken as a group was also statistically examined. The 3 h acid responses to sham feeding with and without secretin infusion were compared. In this analysis the t test formula for paired values was used (Snedecor and Cochran 1967).

Results

Acid secretion

First series 3.0 U of secretin/kg/h significantly ($p < 0.05$) reduced the acid output after 10 min of sham feeding in only 1 of 3 dogs (Table I). 1.5 U/kg/h did not significantly reduce output in any instance. With 0.75 U/kg/h there was a tendency towards *increased* acid outputs. Acid output after 1 min of sham feeding was reduced in 1 of 2 dogs by 3.0 U/kg/h. Acid secretion was not significantly inhibited by this dose of secretin when the same dog (B) was sham fed for 10 min.

Cholecystokinin in doses of 0.75—3.0 U/kg/h did not significantly inhibit the sham feeding evoked gastric acid secretion in any of the dogs (Table II). On the contrary, there was often a tendency towards increased acid outputs when cholecystokinin was infused.

Acid response to sham feeding also tended to increase when caerulein (0.06—0.30 μg/kg/h) was infused (Table II).

3.0 U of secretin/kg/h together with 3.0 U of cholecystokinin/kg/h inhibited the sham feeding response in 1 of 2 dogs (Table III). In this dog (A) inhibition was also produced by 3.0 U of secretin/kg/h given alone.

Second series 2 of 4 antrectomized dogs did not respond to 10 min of sham feeding; the responses in the other two were of moderate size. Subthreshold or

TABLE II Acid responses to 10 min of sham feeding (SF) in 3 Pavlov pouch dogs with open gastric cannulae during concomitant i v infusions of 0 15 M NaCl cholecystokinin or caerulein Acid outputs in response to i v infusions of cholecystokinin and caerulein preceding SF are also given (2nd h of constant infusion)

Dog	Number of expts	Dose of cholecystokinin U/kg/h	Dose of caerulein µg/kg/h	Acid output before SF mEq/h	Acid output after SF mEq/3 h	Change as % of control value	Degree of significance
A	7	0	0	–	2 29±0 35	–	–
	3	0 75	0	0 00±0 00	4 17±1 80	+ 82	p > 0 05
	3	1 5	0	0 00±0 00	1 37±0 71	– 40	p > 0 05
	4	3 0	0	0 17±0 08	1 86±0 49	– 19	p > 0 05
B	10	0	0	–	1 27±0 23	–	–
	3	0 75	0	0 02±0 01	3 18±0 49	+150	p < 0 01
	3	1 5	0	0 00±0 00	2 75±0 81	+117	p < 0 05
	4	3 0	0	0 01±0 01	2 35±0 28	+ 85	p < 0 05
	3	0	0 06	0 02±0 01	2 33±0 45	+ 83	p > 0 05
	3	0	0 30	0 48±0 10	3 86±0 22	+204	p < 0 001
C	9	0	0	–	7 60±0 43	–	–
	3	3 0	0	0 03±0 01	8 61±0 97	+ 13	p > 0 05
	3	0	0 06	0 32±0 13	8 44±1 11	+ 11	p > 0 05
	3	0	0 30	1 27±0 23	8 56±0 29	+ 13	p > 0 05

TABLE III Acid responses to 10 min of sham feeding in 2 Pavlov pouch dogs with open gastric cannulae during i v infusion of secretin and cholecystokinin simultaneously

Dog	Number of expts	Dose of secretin U/kg/h	Dose of cholecystokinin U/kg/h	Acid output mEq/3 h	Change as % of control value	Degree of significance
	7	0	0	2 29±0 35	–	–
	2	1 5	1 5	1 50±0 01	–34	p > 0 05
	2	3 0	3 0	0 17±0 01	–93	p < 0 01
B	10	0	0	1 27±0 23	–	–
	3	3 0	3 0	1 69±0 29	+33	p > 0 05

threshold doses of gastrin markedly enhanced the response to sham feeding These potentiated responses were not significantly ($p > 0.05$) reduced by 3 0 U of secretin/kg/h either in any individual dog (Table IV) or in the dogs as a group

Third series Table V illustrates the responses to sham feeding in 4 antrectomized dogs It should be observed that responses to long periods of sham feeding (30 min) were small

2 antrectomized dogs were sham fed for 1 min during concomitant infusion of secretin (0 24—2 4 U/kg/h) The sham feeding response was not affected by these doses of secretin (Table VI)

TABLE IV Acid response to 10 min of sham feeding (SF) in 4 antrectomized Pavlov pouch dogs with open gastric cannulae during concomitant i.v. infusions of 0.15 M NaCl, gastrin or gastrin plus secretin. Acid outputs in response to i.v. infusions of gastrin plus secretin preceding SF are also given (2nd h of constant infusion)

Dog	Number of expts	Dose of gastrin µg/kg/h	Dose of secretin U/kg/h	Acid output before SF mEq/h	Acid output after SF mEq/3 h	Change as % of control value	Degree of significance
B	2	0	0	—	1.02 ± 0.25	—	—
	2	0.15	0	0.01 ± 0.01	6.42 ± 0.88	—	—
	2	0.15	3.0	0.02 ± 0.01	3.80 ± 0.08	−41	p > 0.05
C	3	0	0	—	0.92 ± 0.32	—	—
	3	0.15	0	0.07 ± 0.04	2.69 ± 0.68	—	—
	2	0.15	3.0	0.12 ± 0.11	2.75 ± 0.07	+ 2	p > 0.05
G	5	0	0	—	0.15 ± 0.05	—	—
	4	0.06	0	0.03 ± 0.01	1.85 ± 0.30	—	—
	4	0.06	3.0	0.02 ± 0.01	1.93 ± 0.25	+ 4	p > 0.05
H	3	0	0	—	0.07 ± 0.06	—	—
	3	0.30	0	0.20 ± 0.05	2.14 ± 0.20	—	—
	3	0.30	3.0	0.04 ± 0.02	1.71 ± 0.13	−20	p > 0.05

TABLE V Acid responses to 1, 10 and 30 min of sham feeding in 4 antrectomized Pavlov pouch dogs with open gastric cannulae

Dog	Number of expts	Period of sham feeding min	Acid output mEq/3 h
D	4	1	0.02 ± 0.01
	2	10	0.22 ± 0.13
	3	30	0.17 ± 0.13
E	4	1	0.10 ± 0.07
	3	30	0.51 ± 0.24
F	7	1	0.08 ± 0.03
	2	10	0.48 ± 0.35
	7	30	0.66 ± 0.36
G	4	1	0.04 ± 0.02
	5	10	0.15 ± 0.05
	3	30	0.15 ± 0.09

TABLE VI Acid responses to 1 min of sham feeding in 2 antrectomized Pavlov pouch dogs with open gastric cannulae with and without concomitant infusions of secretin

Dog	Number of expts	Dose of secretin U/kg per h	Acid output mEq/3 h
D	4	0	0.02 ± 0.01
	2	0.24	0.01 ± 0.00
	2	2.4	0.04 ± 0.01
E	4	0	0.10 ± 0.07
	2	0.24	0.03 ± 0.01
	2	2.4	0.01 ± 0.00

TABLE VII Acid responses to sham feeding (SF) in 4 antrectomized Pavlov pouch dogs with open gastric cannulae during concomitant i v infusions of 0 15 M NaCl cholecystokinin (commercial and pure preparations) or caerulein Acid outputs in response to i v infusions of cholecystokinin or caerulein preceding SF are also given (2nd h of constant infusion)

Dog	Number of expts	Period of SF min	Dose of cholecystokinin U/kg/h	Dose of caerulein μg/kg/h	Acid output before SF mEq/h	Acid output after SF mEq/3 h
D	4	1	0	0		0 02 ± 0 01
	2	1	0 24	0	0 00 ± 0 00	0 04 ± 0 01
	3	1	2 4	0	0 11 ± 0 08	0 91 ± 0 31
	4	1	4 8	0	0 01 ± 0 01	2 15 ± 1 60
	3	1	0	0 012	0 00 ± 0 00	0 54 ± 0 50
	2	1	0	0 06	0 00 ± 0 00	2 57 ± 0 46
L	4	1	0	0		0 10 ± 0 07
	3	1	0 24	0	0 01 ± 0 00	0 02 ± 0 00
	2	1	2 4	0	0 00 ± 0 00	0 69 ± 0 49
	4	1	4 8	0	0 00 ± 0 00	1 88 ± 1 28
	3	1	0	0 012	0 00 ± 0 00	0 08 ± 0 03
	2	1	0	0 06	0 00 ± 0 00	5 20 ± 0 18
F	7	1	0	0		0 08 ± 0 03
	2	1	0 6	0	0 01 ± 0 00	0 36 ± 0 11
	3	1	2 4	0	0 03 ± 0 02	2 71 ± 0 35
	2	1	2 4 (PURE)	0	0 16 ± 0 10	3 01 ± 1 16
G	3	1	0	0		0 04 ± 0 02
	2	1	0 6	0	0 00 ± 0 00	0 03 ± 0 00
	3	1	2 4	0	0 01 ± 0 01	0 25 ± 0 09
	2	1	2 4 (PURE)	0	0 14 ± 0 01	0 45 ± 0 14
	5	10	0	0		0 15 ± 0 05
	3	10	0 6	0	0 02 ± 0 01	0 71 ± 0 21

4 antrectomized dogs were sham fed for 1—10 min during infusion of cholecystokinin (0 24—4 8 U/kg/h) and caerulein (0 012—0 06 μg/kg/h) These agents given alone rarely induced acid secretion (Table VII) When the sham feeding period was 1 min 2 4 U of cholecystokinin/kg/h produced enhancement of the output after sham feeding When the period of sham feeding was prolonged to 10 min in 1 dog 0 6 U/kg/h sufficed to enhance the response No marked difference was found between the enhancing effects of the pure and commercial preparation of cholecystokinin Caerulein (0 06 μg/kg/h) also markedly enhanced the response to sham feeding

Pepsin secretion

First series There was a general tendency towards increased pepsin output after 10 min of sham feeding when secretin was infused (Table VIII) This tendency was statistically significant ($p < 0.05$) but only for the highest dose of secretin (3 0 U/kg/h) All 3 dogs showed a statistically insignificant ($p > 0.05$) reduction of pepsin output following 10 min of sham feeding when the highest dose of chole

TABLE VIII Pepsin output in response to sham feeding (SF) in 3 Pavlov pouch dogs with open gastric cannulae during concomitant i v infusions of 0.15 M NaCl or secretin

Dog	Number of expts	Period of SF min	Dose of secretin U/kg/h	Pepsin output $PU^{Hb}/10^{-}$ /3 h	Change as of control value	Degree of significance
A	4	10	0	2300 ± 800	—	—
	3	10	0.75	5600 ± 1900	+143	$p > 0.05$
	6	10	1.5	6000 ± 2000	+161	$p > 0.05$
B	10	10	0	1300 ± 200	—	—
	3	10	0.75	2200 ± 1200	+ 69	$p > 0.05$
	3	10	1.5	2200 ± 500	+ 69	$p > 0.05$
	4	10	3.0	3900 ± 1300	+200	$p < 0.05$
C	8	10	0	5400 ± 1100	—	—
	3	10	3.0	10000 ± 600	+ 85	$p < 0.05$
	7	1	0	3300 ± 1400	—	—
	3	1	3.0	14800 ± 4300	+348	$p < 0.01$

TABLE IX. Pepsin output in response to 10 min of sham feeding in 3 Pavlov pouch dogs with open gastric cannulae during concomitant i v infusions of 0.15 M NaCl cholecystokinin or caerulein

Dog	Number of expts	Dose of cholecystokinin U/kg/h	Dose of caerulein μg/kg/h	Pepsin output $PU^{Hb}/10^{-}$ /3 h	Change as of control value	Degree of significance
A	4	0	0	2300 ± 800	—	—
	3	0.75	0	4000 ± 1400	+74	$p > 0.05$
	2	1.5	0	2400 ± 1300	+ 4	$p > 0.05$
	3	3.0	0	1600 ± 500	−30	$p > 0.05$
B	10	0	0	1300 ± 200	—	—
	3	0.75	0	2100 ± 400	+62	$p > 0.05$
	3	1.5	0	2200 ± 1100	+69	$p > 0.05$
	4	3.0	0	600 ± 200	−54	$p > 0.05$
	3	0	0.06	2000 ± 500	+54	$p > 0.05$
	3	0	0.30	200 ± 200	−85	$p < 0.05$
C	8	0	0	5400 ± 1100	—	—
	3	3.0	0	4700 ± 200	−13	$p > 0.05$
	3	0	0.06	3300 ± 2200	−39	$p > 0.05$
	3	0	0.30	3300 ± 1400	−39	$p > 0.0$

cystokinin (3.0 U/kg/h) was given (Table IX) The higher dose of caerulein (0.30 μg/kg/h) also seemed to inhibit pepsin output after sham feeding the inhibition was statistically significant only in 1 dog (Table IX)

Second series In all 4 dogs pepsin output after sham feeding was increased by infusion of 3.0 U of secretin/kg/h Statistically significant increases ($p < 0.05$) were found in 3 dogs (Table X

TABLE V Pepsin output in response to 10 min of sham feeding in 4 antrectomized Pavlov pouch dogs with open gastric cannulae during concomitant i v infusion of 0 15 M NaCl gastrin or gastrin plus secretin

Dog	Number of expts	Dose of gastrin μg/kg/h	Dose of secretin U/kg/h	Pepsin output PU^Hb/10^-4/3 h	Change as % of control value	Degree of significance
B	2	0 15	0	5300±1500	—	—
	2	0 15	3 0	7400±1900	+ 40	p > 0 05
C	3	0 15	0	4000± 400	—	—
	2	0 15	3 0	6600± 400	+ 65	p < 0 05
G	4	0 06	0	6400± 600	—	—
	4	0 06	3 0	13300±1700	+108	p < 0 01
H	4	0 30	0	1100± 200	—	—
	3	0 30	3 0	5600±1000	+409	p < 0 05

Discussion

Acid secretion

The present results confirm that the secretory response to sham feedings is dependent upon an intact antral release of gastrin Resection of the pyloric antrum considerably reduces the response to sham feeding Postantrectomy responses to 10 min of sham feeding were usually quite small Subthreshold doses of gastrin markedly enhanced the sham feeding response This agrees with the results of Olbe (1964) and further results of the present author (Sjodin in press *a*)

Johnson and Grossman (1969) have reported that doses of secretin even as low as 0 12 U/kg/h promptly inhibit gastric acid secretion stimulated by doses of gastrin which produce a maximal secretion 2 0 U secretin/kg/h inhibited the secretion by more than 90 % When a lower dose of gastrin was given 4 0 U of secretin/kg/h blocked the secretion completely (Johnson and Grossman 1968) As the secretory response to sham feeding is dependent upon small amounts of gastrin it seemed reasonable to assume that this secretion should also be markedly inhibited by a moderate dose of secretin However even 3 0 U/kg/h reduced the secretion stimulated by 10 min of sham feeding significantly in only 1 of 3 dogs with intact antrum and the potentiating effect of gastrin on the response in antrectomized animals was not inhibited significantly in any of 4 dogs If more experiments had been carried out the acid responses to sham feeding might have been found to be significantly inhibited by secretin in some additional dogs However it should be emphasized that the gastrin potentiated response to sham feeding was not found to be significantly ($p > 0.05$) inhibited by secretin (3 0 U/kg/h) when the results from the group of dogs were pooled It is of course possible that higher doses of secretin would have produced inhibition A lower dose 0 75 U/kg/h produced a tendency towards increased responses in dogs with intact antrum On the other hand 0 24—2 4 U/kg/h produced no effects on the response to sham feeding in antrectomized dogs The tendency towards increased responses in dogs with preserved

antrum might be due to reflux of alkaline pancreatic juice into the antrum there by facilitating the release of gastrin (Andersson and Olbe 1964) Already during infusion of 0 75 U of secretin/kg/h the secretion of the main stomachs was mostly voluminous and of low acidity indicating an efficient stimulation of pancreatic water and bicarbonate secretion

According to Johnson and Grossman (1969) 1 2 U of secretin/kg/h produces a pancreatic bicarbonte response correpsonding to 50 % of the maximum response in dogs The highest dose used in the present experiments (3 0 U/kg/h) is according to Brooks and Grossman (1970) capable of producing a bicarbonate secretion corresponding to approximately 80 % of the maximum Similar results have also been obtained from 1 of the dogs (Dog C) of the present study, when this dog after the completion of the current experiments was provided with a pancreatic fistula It is doubtful if secretin is released in amounts corresponding to 3 0 U/kg/h under physiologic conditions Brooks and Grossman (1970) have shown that the pancreatic bicarbonate response to a meal is only about 10—20 % of the response when the test meal is combined with an infusion of exogenous secretin (3 0 U/kg/h) Therefore it is questionable if secretin in amounts corresponding to those released by a meal has any significant inhibitory effect on gastric secretion evoked by sham feeding

Cholecystokinin inhibits gastric secretion stimulated by endogenous and exogenous gastrin (Gillespie and Grossman 1964 Brown and Magee 1967 Johnson and Grossman 1970) However, it has also been shown that cholecystokinin increases methacholine stimulated secretion (Nakamura Nakajima and Magee 1968) There was no significant inhibition of the sham feeding induced acid secretion in dogs with intact antrum by cholecystokinin On the contrary there was a tendency towards increased acid output This tendency was most marked with the lowest dose 0 75 U/kg/h The increase was in this case probably not caused by reflux of substantial amounts of alkaline duodenal contents since the juice from the gastric cannula in all experiments was of high acidity It has been reported that bile salts are able to release gastrin (Bedi *et al* 1971) Such a mechanism could have been operating in the present experiments as some bile regurgitation into the main stomach frequently took place when cholecystokinin was infused But as cholecystokinin also increased the acid output after sham feeding in antrectomized dogs the increase is probably due to a direct effect of cholecystokinin on the parietal cells

In dogs with resected antrum cholecystokinin in a dose of 2 4 U/kg/h enhances the response to 1 min of sham feeding As shown with gastrin *in another study* (Sjodin in press *a*) prolongation of the period of sham feeding decreases the dose of stimulatory hormone needed for enhancement of the sham feeding effect in antrectomized dogs Even a dose as low as 0 6 U/kg/h which is probably physiologic increased the acid response to 10 min of sham feeding Endogenous cholecystokinin may be of limited importance for the response to vagal stimulation in dogs with intact antrum *but may be important in postprandial secretion in antrectomized* dogs In such dogs cholecystokinin might play the role of an enterogastrin

The commercial preparation of cholecystokinin used in most experiments in the present study has been reported to contain a highly active enterogastrone not identical with cholecystokinin (*Brown et al 1969* Brown Mutt and Pederson 1970) The inhibitory effect of the commercial preparation on gastrin stimulated secretion may be partially caused by this enterogastrone As there was no marked difference in the present investigation between the effects of the commercial and pure preparation of cholecystokinin thus may indicate that the described enterogastrone lacks inhibitory effects on secretion stimulated by sham feeding However this must be confirmed in experiments with the pure preparation of the peptide

The effects of caerulein in the current study were similar to those of cholecystokinin No inhibitory effect on acid secretion evoked by sham feeding could be elicited by 0 06—0 30 µg/kg/h which contrasts to the results with gastrin stimulated secretion which could be markedly inhibited by 0 2 µg/kg/h (Stening Johnson and Grossman 1969) On the contrary the secretory output after sham feeding was often increased by caerulein this was particularly obvious in antrectomized dogs

Pepsin secretion

The present study shows that the pepsin output induced by sham feeding can be further increased by administration of submaximal doses of secretin As the increase in pepsin output was of statistical significance only with the highest dose of secretin used (3 0 U/kg/h) it is uncertain from these experiments if this effect of secretin is of physiologic significance

It is not possible to draw any definite conclusions about the possible influence of cholecystokinin and caerulein on vagally activated pepsin secretion from this study the results were too variable However there was a general tendency towards reduced pepsin output after sham feeding during infusion of a high dose of cholecystokinin (3 0 U/kg/h) or caerulein (0 30 µg/kg/h) In another study (Sjodin in press *b*) it has been found that cholecystokinin (3 0 U/kg/h) inhibits pepsin secretion after a test meal in Pavlov pouch dogs these observations are in good accordance with those of other authors Nakajima and Magee (1970) recently reported that a similar dose of cholecystokinin inhibits methacholine stimulated pepsin secretion Earlier Olbe Ridley and Uvnas (1968) and Vagne and Grossman (1969) have observed that suprathreshold doses of gastrin reduce the pepsin response to vagal stimulation As gastrin cholecystokinin and caerulein have identical c terminal amino acid sequences (Jorpes 1968) mutual effects are probably produced by this part of the molecules Thus not only the acid stimulating but possibly also the pepsin inhibitory effect of these agents are due to their c terminal parts However the physiological relevance of the inhibitory effect on vagally stimulated pepsin secretion induced by these substances remains to be demonstrated

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Influence of Secretin and Cholecystokinin on Canine Gastric Secretion Elicited by Food and by Exogenous Gastrin

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Abstract

SJODIN L *Influence of secretin and cholecystokinin on canine gastric secretion elicited by food and by exogenous gastrin* Acta physiol scand 1972 *85* 110—117

The present study showed that in Pavlov pouch dogs gastrin stimulated acid secretion was more markedly inhibited by secretin and cholecystokinin than the secretion elicited by food. The acid secretory response to a test meal was only moderately reduced by 3 0 U of secretin/kg/h the reduction was not statistically significant In 2 of 3 tested dogs inhibition of the secretion after a test meal was more marked when 3 0 U of cholecystokinin (a 10 % pure preparation)/kg/h was administered However this dose of cholecystokinin caused some side effects such as decreased appetite in the dogs These doses of cholecystokinin and secretin probably produce unphysiologically high blood levels of the hormones In spite of the high doses the acid secretory response to a test meal was never abolished This makes it doubtful if these hormones should be regarded as physiologically important inhibitors of gastric acid secretion Pepsin output after a meal was clearly enhanced by a concomitant infusion of 3 0 U of secretin/kg/h On the other hand the same number of units of cholecystokinin markedly inhibited the pepsin output after a meal

Both secretin and cholecystokinin have been reported to inhibit gastrin induced acid secretion (Greenlee *et al* 1957 Gillespie and Grossman 1964) It has also been reported that the same agents inhibit food stimulated acid secretion (Greenlee *et al* 1957 Nakamura Nakajima and Magee 1968) These studies have however mainly been performed on dogs with denervated pouches It has recently been reported that secretin only causes a minor reduction of the acid output after a meal in Pavlov pouch dogs (Brooks and Grossman 1970) Acid secretion in response to sham feeding has been found not to be regularly inhibited to a significant degree by secretin (Sjodin in press) To what extent cholecystokinin inhibits gastric secretion after a meal in vagally innervated preparations is unknown The secretion evoked by sham

Parts of this investigation were presented at the International Symposium on Gastrointestinal Hormones at the Medical Department University of Erlangen Nuremberg Erlangen 1971

TABLE I Postprandial acid secretion in 3 Pavlov pouch dogs during concomitant infusions of saline or secretin

Dog	Number of experiments	Dose of secretin U/kg/h	Acid output mEq/3 h	Change as % of control value	Significance of change
B	4	0	8.99 ± 1.07	—	
	3	3.0	6.41 ± 1.27	−29	$p > 0.05$
C	4	0	9.40 ± 1.61	—	
	3	3.0	10.41 ± 1.38	+11	$p > 0.05$
I	4	0	4.85 ± 0.76	—	
	3	3.0	3.4[illegible] ± 0.80	−29	$p > 0.05$

Statistical evaluation of data

Mean values are given together with S.E. in the tables and, because of the small number of observations, together with range in the figures. The influences of exogenous secretin and cholecystokinin on the secretory responses to meals were estimated by comparing the 3-hour outputs after meals during secretin or cholecystokinin infusions with the corresponding responses during saline infusions. The t test for unpaired values was used in the statistical analysis (Snedecor and Cochran 1967). The effects of secretin and cholecystokinin on gastrin stimulated acid secretion were calculated by comparing the output during the second (gastrin alone) and third hour (gastrin plus secretin or cholecystokinin) of infusion. Analysis of variance was used in the statistical calculations of these results (Bennett and Franklin 1967).

Results

Only the responses of the pouches are given in this paper since the juice of the main stomachs was often contaminated with bile and pancreatic juice.

Secretion of acid

Series A

In 2 dogs 3.0 U of secretin/kg/h inhibited the acid response to a meal only to a moderate, statistically insignificant degree ($p > 0.05$). In the third dog the same dose of secretin did not reduce the acid output after feeding at all (Table I). Cholecystokinin exerted effects similar to those of secretin in these dogs. However, in

TABLE II Postprandial acid secretion in 3 Pavlov pouch dogs during concomitant infusions of saline or cholecystokinin

Dog	Number of experiments	Dose of cholecystokinin U/kg/h	Acid output mEq/3 h	Change as % of control value	Significance of change
B	4	0	8.99 ± 1.07	—	
	3	3.0	4.35 ± 0.90	−52	$p < 0.05$
C	4	0	9.40 ± 1.61	—	
	3	3.0	10.34 ± 2.12	+10	$p > 0.05$
I	4	0	4.85 ± 0.76	—	
	3	3.0	1.47 ± 0.68	−70	$p < 0.05$

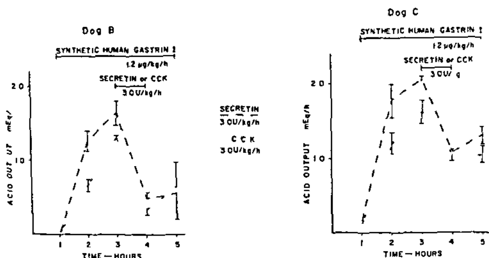


Fig 1 Acid responses in 2 Pavlov pouch dogs (Dogs B and C) with open gastric cannulae during i v infusions of gastrin and secretin or cholecystokinin (CCK) Each symbol represents the mean of 2 expts Vertical bars denote the range in this and the following figures

the former 2 dogs the inhibition was more pronounced with 3 0 U of cholecysto kinin/kg/h than with the same number of units of secretin (Table II) This was particularly marked in dog I It should be emphasized that this dose of cholecystokinin preparation gave some side effects The dogs normally ate their meals in 2—3 min but when given 3 0 U of cholecystokinin/kg/h they often took around 10 min to consume the meal Dog I also frequently had diarrhea when cholecystokinin was ad ministered which was not the case when saline or secretin were infused

Series B

3 0 U of either secretin or cholecystokinin/kg/h inhibited ($p < 0\ 01$) gastrin induced acid secretion (Fig 1) The dose of gastrin used in these experiments (1 20 µg/kg/h) was chosen to give on average around 50 % of the maximal secretion obtained in the dose response experiments with gastrin (Fig 2) Both dogs exhibit a more pro nounced inhibition by secretin and cholecystokinin when gastrin was the stimulus than when the secretion was elicited by a meal

Secretion of pepsin

Series A

All three dogs excreted much more pepsin after a meal when 3 0 U of secretin kg/h was infused than when only saline was given (Table III) even though the increase in one of the dogs was not statistically significant ($0\ 10 > p > 0\ 05$) When 3 0 U of cholecystokinin was given the postprandial secretion of pepsin was reduced in all three dogs However statistically significant reductions were only obtained in two of the dogs (Table IV)

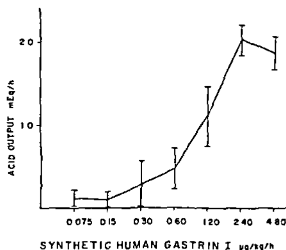


Fig 2 Mean acid responses to graded doses of synthetic human gastrin I in 2 Pavlov pouch dogs (Dogs B and C) with open gastric cannulae Each symbol represents the mean of 4 expts

TABLE III Postprandial pepsin secretion (PU = pepsin units) in 3 Pavlov pouch dogs during concomitant infusions of saline or secretin

Dog	Number of experiments	Dose of secretin U/kg/h	Pepsin output PUHb/10 /3 h	Change as % of control value	Significance of change
B	4	0	3600± 600	—	
	3	3 0	9500±1900	+164	$p < 0 05$
C	4	0	1600± 500	—	
	3	3 0	8500±1400	+431	$p < 0 01$
	3	0	2800± 800	—	
	3	3 0	8400±2200	+200	$p > 0 05$

TABLE IV Postprandial pepsin secretion (PU = pepsin units) in 3 Pavlov pouch dogs during concomitant infusions of saline or cholecystokinin

Dog	Number of experiments	Dose of cholecystokinin U/kg/h	Pepsin output PUHb/10 /3 h	Change as % of control value	Significance of change
B	4	0	3600±600	—	
	3	3 0	1200±200	—67	$p < 0 05$
C	4	0	1600±500	—	
	3	3 0	1000±300	—38	$p > 0 05$
I	3	0	2900±800	—	
	3	3 0	200±100	—93	$p < 0 05$

Series B

Both dogs secreted pepsin poorly in response to 1 20 µg of gastrin/kg/h When secretin was infused concomitantly in a dose of 3 0 U/kg/h there was a tendency towards a rise in the pepsin output (Fig 3) No such tendency was seen when 3 0 U of cholecystokinin/kg/h was infused

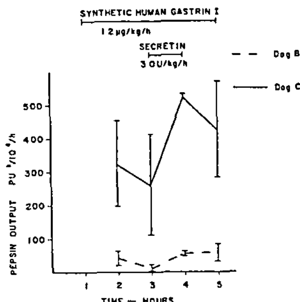


Fig 3 Pepsin responses in 2 Pavlov pouch dogs (Dogs B and C) with open gastric cannulae during infusions of gastrin and secretin Each symbol represents the mean of 2 expts

Discussion

Secretion of acid

The present study shows that exogenous secretin is only moderately effective as an inhibitor of postprandial gastric acid secretion from innervated fundic pouches According to Brooks and Grossman (1970) the dose of secretin used produces around 80 % of the maximal bicarbonate response to secretin This is in accordance with results obtained in one of the dogs (Dog C) in the present study After the completion of the present experiments this dog was provided with a pancreatic fistula, and experiments were performed to investigate dose response relationships for secretin and cholecystokinin Furthermore it has recently been reported that in dogs pancreatic bicarbonate secretion is stimulated to a very limited degree by a test meal compared with the high secretory rate attained when 3 0 U of secretin/kg/h is infused concomitantly with the meal (Brooks and Grossman 1970) This indicates that the blood level of secretin after a meal is considerably lower than during an infusion of 3 0 U/kg/h If so it is reasonable to assume that this dose is higher than physiologic in spite of this the postprandial gastric acid secretion was only moderately inhibited Similar results have also been reported by Brooks and Grossman (1970) who found that the gastric secretion after a test meal was inhibited by 30 % (4 hour output) in Pavlov pouch dogs by the same dose of secretin as used in the present experiments Such results make the suggestion by Johnson and Grossman (1968) that secretin is a physiologically important enterogastrone less likely

In the present experiments gastrin induced secretion was more markedly inhibited by secretin than postprandial secretion The explanation for this may be that the secretory rate induced by gastrin was lower than that elicited by test meals However another series of experiments in Pavlov pouch dogs at this laboratory showed that even acid secretion stimulated maximally by pentagastrin was markedly inhibited by 3 0 U of secretin/kg/h (Sjodin and Elwin unpublished observations) Thus the difference in secretory rates produced by the two stimuli in the present experiments seems to be of only minor significance the difference in the degree of vagal activation which these two stimuli elicit is probably of greater importance During gastrin stimulation it is reasonable to assume that there is only a resting vagal tonus on the parietal cells while these are subject to increased direct vagal stimulation after a meal This is also the case after sham feeding The secretion evoked by sham feeding has been shown to be rather resistant to the inhibitory action of secretin (Sjödin in press)

In the present study cholecystokinin, in a dose of 3 0 U/kg/h, inhibited the response to a meal somewhat more markedly than 3 0 U of secretin/kg/h According to Stening and Grossman (1969) this dose of cholecystokinin produces about 50 % of the maximal pancreatic enzyme response to cholecystokinin This was also later found to be the case for one of the dogs (Dog C) in the present study The pancreatic enzyme response to a meal is about the same as the enzyme output induced by a maximal dose of cholecystokinin (Preshaw Cooke and Grossman 1966 Stening and Grossman 1969) However the high pancreatic enzyme output after a meal might be caused by lower doses of cholecystokinin acting together with gastrin secretin and vagal activity all of which are operating after a meal and all of which are also known to increase the pancreatic enzyme output (Gregory and Tracy 1964 Stening and Grossman 1969 Meyer Spingola and Grossman 1971 Popielski 1896) The side effect decreased appetite observed with the cholecystokinin preparation in the present experiments indicates that the dose used was unphysiologically high However this side effect could of course have been due to impurities in the preparation and not to cholecystokinin itself but in a single test meal experiment with pure cholecystokinin (3 0 U/kg/h) the most sensitive dog (Dog I) exhibited the same side effects as with the commercial preparation. In any case the relatively high dose of cholecystokinin used physiologic or not never abolished the postprandial secretion and did not diminish the secretion at all in one dog As it has also been shown that cholecystokinin enhances the secretion evoked by sham feeding (Sjodin in press) it is doubtful if cholecystokinin is a physiologically important inhibitor of gastric acid secretion When gastrin alone is the major stimulus of gastric secretion, as in the present experiments with exogenous gastrin the inhibitory action of cholecystokinin is more pronounced

The cholecystokinin preparation used has been reported to contain a peptide not identical with cholecystokinin with an inhibitory effect on gastric secretion (Brown *et al* 1969 Brown Mutt and Pederson 1970) To what extent if any this peptide has contributed to the inhibitory effects seen in the present experiments is not known

Secretion of pepsin

The present study confirm the results of others showing that secretin can stimulate pepsin secretion (Pratt 1940 Nakajima Nakamura and Magee 1969) and that cholecystokinin can inhibit the output of pepsin (Nakajima and Magee 1970) Obviously these hormones can also influence the pepsin output when vagal stimuli are concomitantly operating However the present study does not indicate to what extent if any these hormonal effects are of physiological significance

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Acta physiol scand 1972 85 118—125

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Utilization of Yolk Cholesterol in the Developing Chick Embryo

By

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Abstract

JAIN, S K P E LINDAHL and O SVANBERG *Utilization of yolk cholesterol in the developing chick embryo* Acta physiol scand 1972 *85* 118—125

^{14}C 4 cholesterol was injected into fertilized hens eggs after different incubation times The embryos were removed after various times and subjected to autoradiographic examination By tracing the label at subsequent stages of development the time sequence for the utilization of the pre formed yolk cholesterol has been determined Up to the 8th day of incubation the cholesterol from the yolk was homogeneously distributed in the embryo with a slightly higher concentration in the peripheral parts of the spinal cord and in the some areas of the brain Two days later a slight elevation of the liver ^{14}C cholesterol concentration occurred followed by a rapid increase after the 15th day Bile was first secreted on the 11th—12th day The incorporation of ^{14}C cholesterol into the central nervous system was partly restricted after the 8th day From the 15th day hardly any labelled cholesterol was incorporated in the brain and spinal cord a specific blood brain barrier had been established The peripheral nerves however were intensively labelled by the 19th day By this time the adrenals and the gonads contained more labelled cholesterol than other tissues

The incorporation of the yolk cholesterol in the body of the young chick and its retention in different organs have recently been studied by autoradiography (Svanberg 1970 1971) It was shown that in tissues concerned with catabolic processes of cholesterol such as the adrenals and the gonads the pre formed cholesterol was taken up in large amounts The peripheral nerves rapidly incorporated cholesterol from the yolk while the incorporation into the brain and spinal cord was very small The retention of the incorporated cholesterol after two months was by far the greatest in the peripheral nerves followed by the white matter of the brain It was concluded that pre formed cholesterol though present in very high concentrations in blood on hatching was for some reason not utilized in the myelination process of the central nervous system

The present investigation was undertaken in order to examine the utilization of the yolk cholesterol in the chick embryo during successive stages of development especially with regard to the nervous system

Experimental method and materials

Autoradiography according to the Ullberg method (1954) as described earlier (Svanberg 1970) was performed on White Leghorn chick embryos of different ages after injection of ^{14}C-4 cholesterol into the eggs One of the embryos in each group of two was removed from the eggs freed from the extra embryonic membranes embedded in cellulose glue and frozen in acetone cooled with dry ice The other embryo was killed by dropping the egg into the cooled acetone The shell was removed and the embryo together with the white and the yolk was embedded and frozen in blocks Times for injection and removal of the embryos are given in Table I The eggs were incubated at 38 C and 50—60 % relative humidity Injection into the yolks of 2 µCi ^{14}C-4 cholesterol in 0 2 ml emulsion prepared according to Bergstrom and Wintersteiner (1941) was performed through holes drilled in the shells The holes were sealed with melted paraffin The injections were made under sterile conditions

The X ray films (Ilford Industrial G) were exposed at 12 C for about 4 weeks

^{14}C 4 cholesterol (61 7 mCi/mmol) was purchased from the Radiochemical Centre Amersham England

Results

Eggs injected before incubation

10 day old embryos The labelled cholesterol was distributed evenly throughout all tissues with the exception of the spinal cord where slightly more radioactivity was found especially in the peripheral regions (Fig 1 A) Certain areas of the optic lobes appeared less radioactive than others (Fig 1 B)

Eggs injected after 8 days of incubation

10 day old embryos The distribution of ^{14}C resembled that found in embryos of the eggs injected before incubation The only differences were a slightly lower concentration in the brain and spinal cord and a somewhat higher concentration in the liver as compared to the muscles (Fig 2 A and 2 B)

Eggs injected after 10 days of incubation

12 day-old embryos The distribution of ^{14}C-cholesterol was again very similar to that in the 10-day old embryos discussed above The differences between the central nervous system and the muscles as well as between the liver and the muscles were slightly more accentuated than in the embryos injected after 8 days The content of the gall bladder was more radioactive than the liver

Eggs injected after 15 days of incubation

17 day old embryos The liver and the gall bladder content showed very high ^{14}C concentrations (Fig 3) whereas no radioactivity could be detected in the central nervous system The peripheral nerves were labelled to about the same extent as the muscles

19 day old embryos All embryos removed on the day before hatching had almost the same distribution of radioactivity irrespective of the time of injection The most characteristic feature was the very high ^{14}C concentrations in the liver and the bile as well as in the peripheral nerves (Fig 4 and 5) The only discernable difference between the embryos was a gradually diminishing ^{14}C concentration in the brain

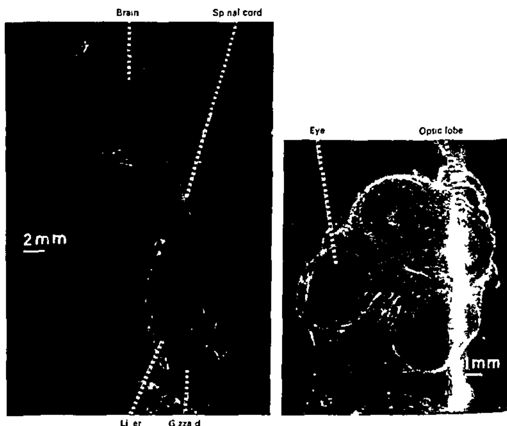


Fig 1 A and 1 B Autoradiographs of 10 day old chick embryos from eggs injected with ^{14}C-4 cholesterol before incubation Note the similarity of the radioactivity in the different tissues of the body and the slight differencs within the optic lobes

TABLE I Chick embryos subjected to autoradiography after injection of C-4 cholesterol at different stages of de elopment

Age of embryo when injected (days)	Age of embryo when removed (days) Number in brackets
0	10 (2)
0	19 (2)
8	10 (2)
8	19 (2)
10	12 (1)
10	19 (1)
15	17 (2)
15	19 (2)

Fig 2 A and 2 B Autoradiographs of 10 day old chick embryos from eggs injected with ^{14}C-4 cholesterol on the 8th day of incubation Note the slightly higher radioactivity (light area) in the liver and the lower activity in the brain as compared to the muscles

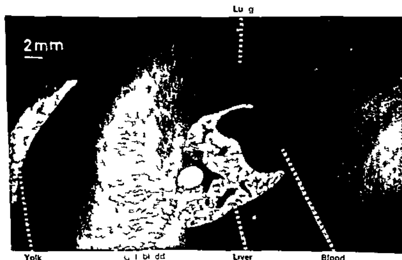


Fig 3 Autoradiograph of a 17 day old chick embryo from an egg injected with ^{14}C-4 [illegible] on the 15th day of incubation Note the high radioactivity (light areas) in the [illegible] and the gall bladder

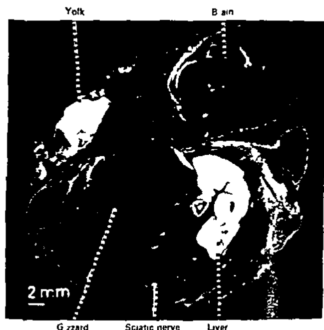


Fig 4 Autoradiograph of a 19 day-old chick embryo from an egg injected with ^{14}C-4 cholesterol on the 8th day of incubation Note the high radioactivity (light areas) in the liver and the sciatic nerve and the much lower activity in the brain

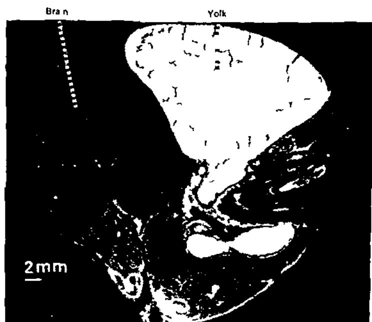


Fig 5 Autoradiograph of a 19-day old chick embryo from an egg injected with ^{14}C 4-cholesterol on the 15th day of incubation Note the absence of radioa tivity (dark area) in the brain.

and spinal cord from eggs injected before incubation to eggs injected after 15 days of incubation The incorporation of labelled cholesterol into the pineal gland was similar to that into other nervous tissues This is contrary to what was found after hatching (Svanberg 1970) The content of the gastrointestinal tract was slightly radioactive in most embryos (Fig 4) The adrenals and the gonads contained more radioactivity than the muscles

Discussion

The ^{14}C-4-cholesterol was injected into the yolk for it to mix with the pre formed cholesterol present The distribution of the label was then considered to correspond to that of the yolk cholesterol

During the first 8 days of incubation the yolk cholesterol was obviously utilized to the same extent throughout the whole embryo After this time the concentration of ^{14}C-cholesterol in the liver gradually rose compared to that in the muscles After the 15th day there occurred a heavy incorporation of pre formed cholesterol from the yolk into the liver This coincides with the rapid increase of the cholesterol concentration in the liver starting at this time and continuing up to about 2 days after hatching (Entenman Lorentz and Chaikoff 1941 Davison *et al* 1958 Moore and Doran 1962 Feldman and Grantham 1964) The present experiments directly demonstrate the exogenous origin of the liver cholesterol deposits as proposed by these workers

Kuo (1932) stated that bile was secreted in 7 to 9-day-old chick embryos In the present experiment bile was secreted into the gall bladder in the 12 day old embryo Whether the bile at this stage contains bile acids or whether the radioactivity originates from secreted cholesterol is not known Whitehouse *et al* (1962) however found measurable cholesterol oxidase activity in liver tissue from 12 day-old chick embryos It could not be ascertained from the autoradiographs whether any bile had been brought into the intestinal canal The slight radioactivity present in the gut may well have arisen from swallowed material The intestinal wall however was rather heavily labelled in the oldest embryos The very strong radioactivity in the lining of the gizzard of the young chick as noted by Svanberg (1971) was not observed

The high concentration of ^{14}C in the adrenals and gonads towards the end of the incubation period probably merely reflects the cholesterol concentration in these organs (Svanberg 1971) which at least in the adrenals is increasing at this time (Mazina 1962)

The impedance offered by the brain to the entry of certain substances from the blood often referred to as the blood brain barrier depends on many individual factors It has been a subject of debate as to whether or not it is a phenomenon which develops during the ontogenesis (for a review of this point see Dobbing 1968) In the present experiment it is shown that the entry of pre formed cholesterol into the central nervous system is partially restricted from about the 8th day of

incubation The hindrance regarded as a specific cholesterol blood brain barrier obviously develops gradually and is fully established towards the end of the second week of incubation

In the spinal cord the neuroblast formation ceases at about the 8th or 9th day Cell proliferation after this time gives rise to neuroglial cells (Bensted *et al* 1957 Fujita 1963) Neuroblasts are also formed later in the brain and separate phases of proliferation for neurons and glial cells are not found (Meller Breipohl and Glees 1966, Glees and Meller 1968) The appearance of the blood brain barrier or blood central nervous system barrier for cholesterol cannot therefore be correlated to proliferative activity

By the 12th day occasional myelin sheaths have been observed in the ventral column of the spinal cord spreading later in a dorsal direction (Bensted *et al* 1957) These authors show that the myelination process is still in progress at hatching Thus the onset of the myelination coincides fairly well with the almost total exclusion of pre formed cholesterol from the spinal cord This indicates that the cholesterol of the myelin at all stages is synthesized *in situ* Since the growth of neurons continues up to the adult stage it would seem that there is also a change from the use of pre formed cholesterol to a *de novo* synthesis in these cells

Myelination starts late in the peripheral nerves The sciatic nerve for example does not contain medullated fibres until the 15th day of incubation (Romanoff 1960) In the present experiment the peripheral nerves could first be observed in 12 day old embryos The ^{14}C cholesterol concentration at this stage was similar to that of the muscles By the 19th day there was a marked accumulation of ^{14}C in the sciatic nerve as was reported for newly hatched chicks (Svanberg 1970) The difference between the central nervous system and the peripheral nerves with regard to the use of exogen cholesterol in myelin formation appears therefore before hatching

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Facilitation and Inhibition of Facial Reflexes in the Cat Induced by Peripheral Stimulation

By

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Abstract

LINDQUIST CHR *Facilitation and inhibition of facial reflexes in the cat induced by peripheral stimulation* Acta physiol scand 1972 85 126—135

The effects of conditioning peripheral stimuli on reflexes elicited in m caninus and m orbicularis oculi by tap stimulation of the facial skin have been studied in cat Following a conditioning facial skin tap the reflex transmission was facilitated for some 20 ms and this facilitation was succeeded by a period of reflex depression lasting up to 400 ms A similar type of interaction was observed when the conditioning stimulus consisted of afferent volleys in the lingual or the hypoglossal nerve Reflex depression of several hundred ms duration resulted when conditioning volleys were elicited in group II afferents in the radial nerve or in afferents of the saphenous nerve —I v injections of strychnine resulted in considerably diminished inhibitory effects of conditioning facial taps which was taken to indicate that postsynaptic mechanisms are involved in the inhibitory reflex control The reflex depression induced by activity in radial nerve afferents was not affected by strychnine injections to the same extent this suggested that the time course of the facial reflex inhibitions indicates involvement of presynaptic inhibition as well —In the chloralose anesthetized cat the blink reflex was suppressed to a considerable degree when elicited at a frequency of 4/s and was almost abolished at 10/s This "reflex fatigue" was not changed by decerebration It is suggested that presynaptic inhibitory mechanisms account for the fatiguability of the blink reflex

Mechanical stimulation of various parts of the facial skin sets up reflex contractions in certain facial muscles in man and cat A typical reflex in the orbicularis oculi or in a perioral muscle is composed of two successive discharges both resulting from activation of mechanoreceptors in the facial skin (Lindquist and Martensson 1970 Shahani 1970) In both species a reflex is most readily evoked by tapping on the skin overlying the muscle and the longer the distance between stimulus site and muscle the stronger is the tap stimulus required (Kugelberg 1952 Lindquist and Martensson 1970) Facial reflexes may even be elicited by activation of nociceptive afferents of the body and the limbs (Gandiglio and Fra 1967 Hanson and Widen 1970) In the cat facial reflexes may also be set up from nociceptive tongue afferents (Lindquist and Martensson 1969 a Hanson and Widen 1970) To a certain extent facial reflexes are thus nociceptive reactions Facial mechanoreceptors might also exert a

proprioceptive reflex control of facial muscles due to the intimate coupling between facial muscle and skin (Lindquist and Mårtensson 1969 b 1970)

Hagbarth (1952) has shown that skin afferents in the hind limbs have inhibitory as well as excitatory reflex connections to flexor and extensor muscles There is a similar relationship between afferents from the thoracic skin and expiratory and inspiratory intercostal muscles (Sumi 1963) In a previous paper (Lindquist and Mårtensson 1970) it was shown that facial nerve afferents of unknown but possibly nociceptive origin may either facilitate or inhibit the cat s blink reflex In the present series of experiments facilitatory and inhibitory influences on facial skin reflexes were studied more closely using mechanical stimulation of different facial skin areas preceded by conditioning stimuli to peripheral nerves or to the facial skin No particular attempts were made to distinguish between conditioning effects on the early and the late components of the facial muscle reflexes

Some aspects on blink reflex fatigue will also be reported

Methods

Experiments were performed on tracheotomized cats weighing 2 to 4 kg Anesthesia was induced by ether and maintained by injections of chloralose in doses of 60—80 mg/kg b wt administered through a catheter in the femoral vein In some experiments suxamethonium (Celocurin®) or gallamine triethiodide (Flaxedil®) was given i v to achieve complete muscle relaxation The cat was kept warm by an infrared heating lamp

Tap stimuli of 3—5 ms duration were delivered by a mechanical stimulator triggering the oscilloscope sweep at the start of movement The moment when contact was made with the skin was not recorded and hence latency measurements may be inaccurate by a few ms

Other experimental procedures and the recording equipment used were described in earlier papers (Lindquist and Mårtensson 1969 a, 1970)

The anatomical nomenclature used in the text is that of Reighard and Jennings (1961)

Results

Effects of conditioning stimuli on tap elicited reflexes In an initial series of experiments the interaction of two tap stimuli applied to facial areas were studied and Fig 1 A—C illustrates an experiment of this type when recording from the orbicularis oculi muscle Taps were delivered to the areas marked *a* and *b* in the inset drawing of the cat s face The left hand record in *A* shows a reflex discharge in response to a light tap to area *a* and the right hand record the discharge set up by a tap to area *b* In the middle is the response obtained on simultaneous tapping of both areas as seen facilitation results in this case In *B* a reflex discharge elicited by tapping area *a* is shown to the left the right hand record results when the tap to *a* is preceded by a *conditioning tap to b by 80 ms The conditioning results in a reduced* reflex amplitude In *C* a test tap to area *a* (left hand record) give a double reflex discharge When as in the right hand record a conditioning tap is delivered to *b* 450 ms prior to the test tap only the first of the two reflex components is elicited the late component being abolished In other experiments with similar long conditioning test intervals the initial reflex component was depressed as well

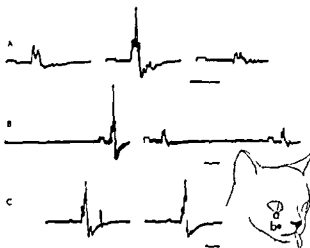


Fig. 1. Reflex discharges in cat's orbicularis oculi muscle produced by taps on areas marked *a* and *b* in inset. *A*, responses set up by tapping on *a* (left) and *b* (right). In the middle facilitation induced by simultaneous tapping on both areas. *B*, test response produced by tapping on *a* (left) is depressed when trailing a conditioning ipsilateral tap to *b* by 80 ms (right). *C*, double reflex response evoked by tapping on *a* (left); when conditioning tap is delivered to *b* 150 ms in advance only the first of the two reflex components is elicited (right). Conditioning stimulus artefact off the sweep in *C*. Time bars 10 ms.

The recordings in Fig. 2*A*—*D* from a similar type of experiment were obtained from the caninus muscle on tapping of the two facial areas marked in the inset. The large reflex response to the left in *A* is reduced to a few discharges in the right hand record when the test tap is trailing a conditioning tap by 10 ms. *B*, *C* and *D* illustrate similar depressing effects of conditioning tap stimuli with conditioning test intervals of 80, 200 and 300 ms respectively.

When the intervals between two stimuli to the ipsilateral half of the face were less than 20 ms facilitation of the reflexes usually resulted, whereas with intervals between 40 and 150 ms reflexes of lower amplitude were obtained. Maximal reflex depression occurred 10 to 80 ms after the conditioning stimulus.

In good preparations similar influences of conditioning taps were noted also when recording the reflex in the contralateral orbicularis oculi. A contralateral conditioning effect is demonstrated in Fig. 3.

The thresholds for evoking reflex facilitation and/or inhibition from a particular facial area were often lower than those for evoking distinct reflex contractions.

Facilitation and inhibition of facial muscle reflexes resulted also when the conditioning stimulus consisted of electrical stimulation of peripheral trigeminal nerve branches. It could thus be excluded that the reduced test reflex response was due to a persisting skin deformation caused by the conditioning tap.

The remarkably long duration of the reflex depression might also be the result of a second inhibitory impulse volley from mechanoreceptors in the skin evoked by a reflex muscle contraction set up by the conditioning tap (*cf.* Lindquist and Mårtensson 1970). To rule out this possibility the experiments described above were repeated using curarized animals and recording the reflex responses from the respective muscle nerves. Also under these experimental conditions the inhibition was however maintained for several hundred ms.

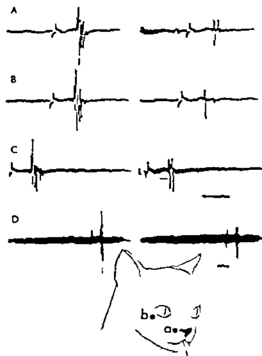


Fig 2 Reflex discharges in cat's caninus muscle produced by taps on areas marked *a* and *b* in inset *A* large amplitude test response set up by tapping on *a* (left) is reduced to a few discharges when trailing a conditioning ipsilateral tap to *b* by 40 ms (right) *B* *C* and *D* similar depressing effects with conditioning test intervals of 80 200 and 300 ms respectively Conditioning stimulus artefact off the sweep in *B* *C* and *D* Same sweep speed in *A* *B* and *C* Time bars 10 ms

In experiments on non anesthetized cats Tokunaga *et al* (1958) found that decerebration at the level of the superior colliculus lowered the threshold for the early component of the blink reflex and abolished the late component More recent investigations have revealed that there exists a cortical inhibitory control of impulse transmission from trigeminal primary afferents (Stewart and King 1966 Wiesendanger *et al* 1967) In view of these findings it was considered to be of interest to study conditioning effects on facial reflexes in decerebrate preparations as well No appreciable changes in the effects of conditioning tap stimuli on the tap-elicited short latency reflexes were however observed in experiments performed on chloral

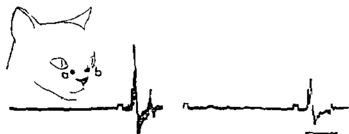


Fig 3 Reflex discharge in cat orbicularis oculi muscle in response to ipsilateral test tap to *a* (left) is depressed when trailing a conditioning contralateral tap to *b* by 0 ms (right) Time bar 20 ms

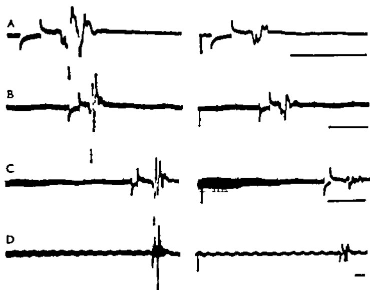


Fig 4 Reflex discharges in cat's orbicularis oculi muscle produced by tap stimulation of medial angle of ipsilateral eye *A* test response (left) is depressed when trailing a conditioning electrical stimulation of ipsilateral radial nerve by 3 ms (right) *B* *C* and *D* similar depressing effects with conditioning test intervals of 30 65 and 300 ms respectively Time bars 20 ms

ose anesthetized cats after collicular decerebration It is thus evident that the inhibitory influences are not dependent on supracollicular structures nor do the experiments suggest any appreciable tonic supracollicular influence on the excitatory and inhibitory pathways to the facial nucleus in the anesthetized cat

As mentioned in the introduction facial reflexes may be obtained also following nociceptive stimulation of the skin in other areas than the face. Another series of experiments was therefore performed in order to study the effects of conditioning volleys in the lingual hypoglossal radial and saphenous nerves on reflexes evoked in facial muscles by tapping the facial skin In these experiments effects were obtained *with stimulus intensities that were below threshold for the facial muscle reflexes* from these nerves Conditioning shocks to lingual or hypoglossal nerve afferents gave facilitatory or inhibitory effects similar to those outlined above and of the type previously obtained on conditioning stimulation of facial nerve afferents (Lindquist and Martensson 1970) Inhibitory effects of fairly long duration were more readily obtained than facilitatory effects Conditioning stimuli to the saphenous nerve gave a long lasting depression of the ipsilateral blink reflex. Afferents in this nerve were recently shown to depress reflexes elicitable in the quadratus labii superioris muscle on stimulation of hypoglossal afferents (Hanson and Widen 1970) Fig 4 *A–D* illustrates an experimental situation in which conditioning of the blink reflex is induced by stimulation of the ipsilateral radial nerve at four different conditioning test intervals The blink reflex is distinctly depressed at an interval as brief as 3 ms (*A*) and the *inhibition is still clearly visible at 300 ms interval* (*D*) The strength of the



Fig 5 Reflex discharges in the zygomatic branch of the facial nerve in a curarized cat 2 min after i v injection of 1 3 mg picrotoxin per kg b wt (*A* and *B*) and 1 min after i v injection of 0 2 mg strychnine per kg (*C*) *A* response following test tap to *a* (left) is still inhibited when trailing a conditioning tap to *b* by 50 ms *B* similar inhibitory effect persists when conditioning test interval is 80 ms *C* after injection of strychnine response to test tap to *a* (left) is no longer appreciably reduced by a conditioning tap to *b* preceding test tap by 40 ms (right) Time bars 20 ms

stimulus applied to the radial nerve in this case is roughly 1 5 times that needed to elicit perceptible contractions of the fore paw muscles The stimulus strength required and the latency of the inhibitory effect indicate that inhibition is produced by activity in group II afferents

Inhibitory mechanisms In another series of experiments attempts were made to find out whether the inhibition of the blink reflex is of pre or postsynaptic type For this purpose the experimental animal was curarized and the reflex activity recorded from the zygomatic branch of the facial nerve Intravenous injections of picrotoxin (max 2 3 mg/kg b wt) and strychnine (max 2 mg/kg) were used to block pre and postsynaptic inhibition respectively As previously described by Tokunaga *et al* (1958) both drugs promptly resulted in an appreciable reduction of the threshold for elicitation of the blink reflex In Fig 5 *A*—*C* the zygomatic nerve recordings illustrate the effects of picrotoxin and strychnine on the interaction of two tap stimuli applied to the facial areas shown in the inset In *A* 2 min after the injection of 1 3 mg picrotoxin per kg a conditioning tap preceding a test tap by 50 ms still results in depression of zygomatic nerve reflex activity Reflex inhibition also results at a conditioning test interval of 80 ms (*B*) 150 ms and 300 ms Despite the occurrence of convulsive nervous activity picrotoxin had no obvious effect on the reflex inhibition during the first hour after the injection Two hours after the picrotoxin injection 0 2 mg strychnine per kg b wt was injected and the recordings in Fig 5 *C* were obtained The record to the left shows a large reflex response to an unconditioned tap stimulus indicating a lowered reflex threshold In the right hand record the test tap is trailing a conditioning tap by 40 ms and although a large reflex is already produced by the conditioning tap there is now no longer any appreciable inhibitory effect on the test reflex The strychnine seems to have blocked the inhibitory mechanism This may indicate that under normal circumstances the conditioning tap depresses the reflex activity by a postsynaptic inhibitory mechanism

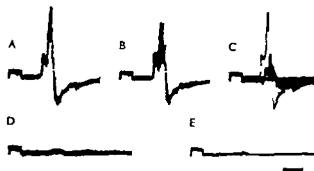


Fig 6 Recordings illustrating blink reflex fatigue induced by tap stimulation of the skin overlying the orbicularis oculi muscle at various stimulus frequencies viz. *A* 0 3 *B* 1 *C* 4 *D* 8 and *E* 10 per s Time bar 5 ms

A similar type of analysis was made of the mechanisms behind the blink reflex depression induced by afferent volleys in the ipsilateral radial nerve A previously subthreshold radial nerve stimulus evoked a large zygomatic nerve reflex response after strychnine injection The latency of this response was 10 ms However the inhibitory effects persisted even after injection of more than 0 2 mg strychnine per kg which has been claimed to block virtually all postsynaptic inhibition in the spinal cord (Bradley *et al* 1953) Picrotoxin did not produce any clear-cut changes in the conditioning effects The findings indicate that the inhibitory mechanisms activated by facial tap stimulation may differ from those activated by radial nerve stimulation

Blink reflex fatigue In man habituation of the two components of the blink reflex occurs at stimulus frequencies ranging from 1 to 4/s (Rushworth 1962 Shahani 1970) This dependence of reflex amplitude on frequency of stimulation has been attributed to cortical inhibitory influences hence the designation habituation Fatigue of the flexor reflex at similar stimulus frequencies has however been ascribed to spinal presynaptic inhibition (see *e g* Schmidt and Willis 1963) In an attempt to throw some light on the mechanism behind the habituation of the human blink reflex some experiments were carried out to study the fatiguability of the blink reflex in anesthetized cats An experiment of this type is illustrated in Fig 6 *A*—*E* showing a series of superimposed tracings of blink reflex discharges produced by repeated tapping on a single facial spot The tapping had been going on for some time when the film was exposed In *A* the reflex is elicited once every 3 s in *B* once per s and as seen the discharge amplitude is the same following each tap In *C* the frequency is 4 taps per s and of the five superimposed tracings only one has the same amplitude as those in *A* and *B* In *D* and *E* at 8 and 10 taps per s respectively the blink reflex is virtually abolished The critical frequency above which the discharge amplitude was rapidly reduced seemed to be 2 to 4 taps per s which means that reflex fatigue can occur when the stimulus intervals are below 400 ms. This limit agrees well with the upper limit at which the test reflexes were depressed in the conditioning test experiments described above Reflex fatigue and the observed depression of the test reflexes may thus be expressions of the same inhibitory

mechanisms If this holds true decerebration should have no effect on fatigue As a matter of fact it had not and hence fatigue of the blink reflex is not dependent on cortical inhibitory influences at least as far as the anesthetized cat is concerned

At a stimulus frequency of 8/s the reflex amplitude declined to a minimum even after 2—3 taps and this low discharge amplitude then persisted for minutes At a frequency of 3—4 taps per s the amplitude was varying in response to the first 5 or 6 taps and was then stabilized below the initial level Regardless of the previous stimulus frequency a full amplitude blink reflex could be obtained following a rest period of a second or so even if the stimulation had been going on for 30 min Effects of longer periods of stimulation were not investigated

The fatigue of the blink reflex was not caused by mechanical adaptation in the skin since it occurred also when the reflex was elicited by electrical stimulation of trigeminal nerve branches nor could it be attributed to fatigue in the neuromuscular junction since it set in also when reflexes were recorded in the muscle nerve

Discussion

The polysynaptic facial muscle reflexes seem to be functionally related to the spinal withdrawal reflexes but whereas the latter have been extensively studied the former have received little attention Reflex transmission to flexor muscles in the spinal cat becomes facilitated a few ms after application of a conditioning stimulus to a nerve from the skin and this facilitation may be sustained for about 40 ms In other cases it may be substituted 20—30 ms after the conditioning stimulus by a more long lasting depression (Hagbarth 1952 Fig 6 and 7) These conditioning effects are thus similar to the conditioning effects from skin afferents on facial reflexes The depression of flexor muscle reflexes can be partly ascribed to postsynaptic inhibition of the flexor motoneurons (see *e g* Holmqvist and Lundberg 1961) In addition presynaptic inhibition of hind and fore limb flexor reflexes lasting 400—800 ms may be evoked by single stimuli applied to various nerves in the limbs (Schmidt and Willis 1963) The strychnine sensitivity of the inhibition of facial reflexes induced by conditioning facial tap stimulation indicates that postsynaptic inhibition can account for at least the early part of the inhibition Results to be presented in a following paper suggest that this postsynaptic inhibition occurs in the facial motoneurons (Lindquist 197?) It is more doubtful whether postsynaptic inhibition can explain also the late part of the tap elicited inhibition and the results presented above do not suggest that this inhibitory mechanism is involved in the facial reflex depression caused by afferent volleys in the radial nerve The long duration of these inhibitory processes rather suggests a presynaptic mechanism It has previously been suggested that presynaptic inhibition is of importance for the control of reflexes set up from various cranial nerves (Wiesendanger *et al* 1967 Sauerland and Mizuno 1970 Lindquist and Martensson 1971)

If presynaptic inhibition is of importance for the facial muscle reflex control trigeminal and radial nerve afferent volleys should be expected to be capable of

inducing depolarization of trigeminal afferent terminals in the brain stem since depolarization of the central terminals of primary afferent fibers is the way by which presynaptic inhibition is generated. This has also been found to be the case (Darian Smith 1965, Baldissera *et al.* 1967, Stewart *et al.* 1967, Vyklicky *et al.* 1967). It has also been pointed out that there is good correlation between the time course of the trigeminal afferent depolarization and the depolarization of other cranial nerve afferents on the one hand and of the depression of reflexes in certain muscles innervated by cranial nerves on the other hand (Wiesendanger *et al.* 1967, Sauerland and Mizuno 1970, Lindquist and Martensson 1971). A possible morphological substrate for the presynaptic control of trigeminal afferents has been found with the aid of the electron microscope (Hassler and Bak 1970).

There is thus indirect support for the concept that presynaptic inhibition plays a part in the control of facial muscle reflexes. However, the effect of picrotoxin was not clear cut as it was in abolishing inhibition of laryngeal and palatal reflexes (Lindquist and Martensson 1971). According to a recent report (Besson *et al.* 1971) picrotoxin may however enhance rather than diminish presynaptic inhibition induced by stimulation of certain loci in the brain. It seems likely that both post and presynaptic inhibitory mechanisms are involved in the control of facial muscle reflexes as well as of flexor reflexes.

The findings reported above were achieved in experiments on chloralose anesthetized cats and hence it has to be taken into account that a prolonged hyperpolarization may set in after reflex activation since this occurs in spinal flexor motoneurons under chloralose anesthesia (Devanandan *et al.* 1969). The cats used in their study demonstrating this type of hyperpolarization received a dose of chloralose optimal for seizure activity. The cats used in the experiments described above were however given larger amounts of chloralose and displayed minimal seizure activity.

In the present investigations no differences in conditioning effects were observed between decerebrate cats and animals with intact brain. It is however very likely that there exists a suprasegmental control of the facial reflexes similar to that governing the spinal flexor reflexes. Tokunaga *et al.* (1958) observed that the long latency component of the blink reflex was abolished on decerebration. This disappearance seems more likely to be an expression for a differential suprabulbar control of the two reflex components than for an actual interruption of the long latency reflex pathway as suggested by them. Shahani (1968) noted a differential effect of sleep on the two components of the blink reflex in man. The findings in the present study give no clues as to the steering function of higher order nervous centers on facial reflexes but it is interesting to note that a reflex is set up from radial nerve afferents on strychninization. Like the Babinski sign the palmomental reflex in man has been reported to occur more frequently in individuals with lesions of motor centers in the brain (Dalby 1970). In the light of the present experiments it seems possible that the palmomental reflex is produced by release of suprabulbar postsynaptic inhibition.

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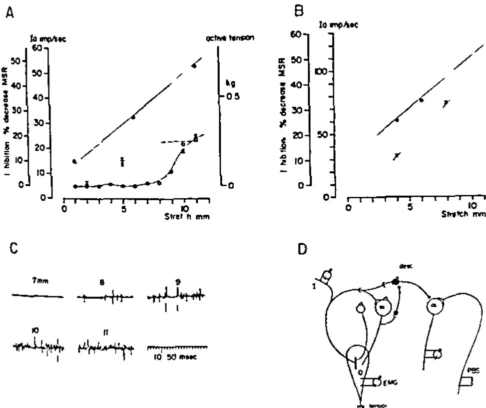


Fig 1 Graphs A and B are from two different experiments in which recordings were made of Ia impulse frequency of a Q afferent (●) the monosynaptic test reflex (MSR) in the L7 and S1 ventral roots on stimulation of the PBSt nerve (x) and Q muscle tension (O) The experimental arrangement is shown in D The graphs give the mean of 4 responses in A (the MSR was exceptionally stable) and 10 responses in B measured during maintained extension to lengths given in abscissa Measurements were made during periods starting 1 5 s after completion of the dynamic phase of the stretch the PBSt nerve was stimulated at 2/s Zero on the abscissa indicates the length below which the Ia impulse frequency did not decrease Inhibition is the decrease of the amplitude of the MSR in % of the MSR at zero length A is from an experiment in which a weak stretch reflex was evoked as revealed by the tension curve (passive tension after section of Q nerve subtracted) and the EMG records in C recorded at the indicated extensions B is from an exceptional experiment in which no stretch reflex was evoked (no EMG activity in Q) despite a high static -bias indicated by the relation between Ia impulse frequency and muscle length

control was observed to operate effectively in all the present experiments While reflex pathways from group I afferents to primary afferent terminals are not tonically inhibited in the decerebrate state we will disregard the effect of presynaptic inhibition evoked from the Q muscle in this initial interpretation of the results

To monitor tension a strain gauge was connected in series with the tendon and recordings were also made of the EMG activity in Q and the firing pattern of a Ia afferent from Q The graphs in Fig 1 give mean static Ia and tension responses during maintained graded extension after completion of the dynamic phase of the

stretch Graph A is from an experiment in which a weak stretch reflex was present while B is from an exceptional experiment in which the stretch reflex was absent Both graphs reveal the expected linear relationship between extension and Ia frequency (●) Graph B and the initial extension in graph A involves a linear relationship between Ia firing rate and reciprocal inhibition (x) With the appearance of a static stretch reflex at 7—8 mm of extension ((○) however a plateau appears in the inhibitory curve while both tension and EMG records (C) show the stretch reflex to increase with further extension In preparations with a more vigourous stretch reflex the inhibitory curve levelled off when the stretch reflex appeared but with increasing active tension at greater lengths the reciprocal inhibition continued to increase Decreasing inhibition was never encountered The plateau in graph A suggests that at different levels of muscle extension the increments in Ia excitation and recurrent inhibition almost exactly balance each other—presumably keeping the frequency of the discharge in the Ia inhibitory interneurones constant

For an interpretation of the increasing reciprocal inhibition during vigourous stretch reflexes it is relevant that Grillner and Udo (1971) have shown a highly alinear recruitment of motoneurones during good stretch reflexes in soleus At increasing lengths there was a successively reduced recruitment of motoneurones and the linear relation between length and tension at greater lengths was largely due to the stiffness of the already active fibres Accordingly we would expect a disbalance between the two opposing processes controlling the Ia inhibitory interneurones at increasing muscle lengths The linear increase in Ia excitatory action would be met with a successively decreased increment in recurrent inhibition the outcome being an increasing reciprocal inhibition at more extreme extension

From the present experiments it is not possible to make any quantitative interference regarding how Ia impulses and recurrent inhibition interact in the control of reciprocal inhibition during load compensation Our observations are nonetheless mpatible with the view that recurrent inhibition may have the function of regulating the depth of reciprocal inhibition in movements depending on α γ linkage It has previously been postulated (Hongo *et al* 1969) that higher centres commanding an α γ linked reciprocal movement can achieve a coupling between excitation and inhibition by evoking excitatory action (dashed lines in Fig 1 D) not only in agonist α and γ motoneurones but also in Ia inhibitory interneurones to antagonist α motoneurones (α , linked reciprocal inhibition) To this scheme may now be added a segmental autoregulatory mechanism that prevents reciprocal inhibition from getting too deep during increased activity in agonist α motoneurones

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From the Department of Anatomy University of Helsinki Finland

Adrenergic and Nonadrenergic Axons of the Rabbit Uterus and Oviduct

By

ANTTI HERVONEN and LASSE KANERVA

After the discovery of both adrenergic and cholinergic axons and nerve terminals in close contact to each other (for ref see Sporrong 1970) the hypothesis of Burn and Rand (1965) was seen in a new light The axo-axonal interactions between the peripheral adrenergic and cholinergic terminals were suggested to be more likely than cholinergic mechanism within the adrenergic axon (Ehinger *et al* 1969) A combination of 2 methods the fluorescence method for catecholamines and the histochemical demonstration of acetylcholinesterase (AChE) (Eranko and Harkonen 1964) revealed that both adrenergic and cholinergic components were often present in the same nerves (Eranko and Raisanen 1965 Jakobowitz and Koelle 1965) The presence of AChE in the nerves of the vas deferences has been demonstrated also by means of electron microscopy (Burnstock and Robinson 1967 Burnstock 1970)

Recently the previously widely neglected studies on the neuronal control of the female reproductive organs have been accelerated (for ref see Marshall 1970) To understand the nature and details of the complex neuroendocrine interactions at the peripheral effector sites in the female reproductive organs the fine structure and histochemistry of the autonomic nerves in this region should be elucidated The present studies were undertaken to find out whether the morphological signs of the axo axonal interactions between the two types of autonomic nerves are present in the rabbit myometrium

The isthmic region of the rabbit oviduct and small pieces of different parts of the uterus were fixed with 3 % $KMnO_4$ according to Hokfelt and Jonsson (1968) For details of the procedure see Hervonen Kanerva and Teravainen (1972)

The terminal nerve fibres corresponding to the fluorescent varicose nerves found by Owman and Sjoberg (1966) contained usually 2—10 axons The varicosities were of 2 types (1) Adrenergic terminals containing small granular vesicles (diam 300—600 Å) and large granular vesicles (diam 800—1100 Å about 2—4 % of the total count of the vesicles) (2) Non adrenergic terminals containing only small agranular vesicles (diam 300—600 Å) (Fig 1) Approximately 10 % of the axons belong to the latter group Non adrenergic axons were not present in all terminal fibres but were with certainty demonstrated in the majority of them The two types of axons or nerve terminals were often found to be in close contact to each other (Fig 2) but only the adrenergic type of terminals was found to form neuromuscular contacts

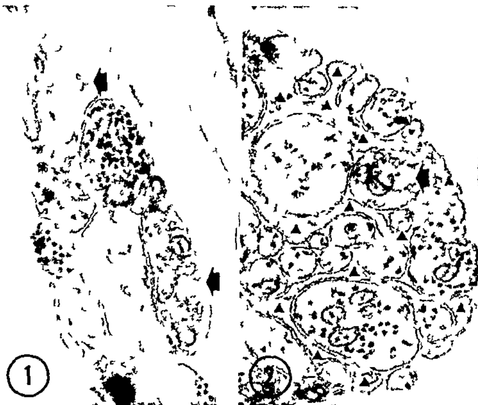


Fig 1 Tangential section of a terminal nerve fibre in the circular muscle coat of the isthmic gion of the rabbit oviduct The cross section of the non adrenergic axons (arrows) are filled h small (diameter 300—600 Å) agranular vesicles while the adrenergic varicosities are sely packed with granular vesicles of the same size ×34 440

Fig 2 Cross section of a larger autonomic nerve from the ground plexus outside the circular muscle layer The adrenergic axons contain richly granular vesicles while the non adrenergic one (arrow) contains only agranular vesicles The cytoplasmic pdocesses of the Schwann cell are marked with triangles Note the lose relation between the adrenergic and non adrenergic axons ×34 440

To our knowledge attempts to differentiate the type of nerve terminals of female reproductive tract have not been performed before with electron microscope The results of Owman and Sjoberg (1966) and Jordan (1970) are in disagreement with the present ones These authors could not find terminal network of AChE positive fibres in the rabbit oviduct with light microscopy Although the non adrenergic axons found in close contact with the adrenergic ones showed typical characteristics of cholinergic nerves they could not be considered cholinergic because of the discrepancy mentioned Instead the term non adrenergic is used to emphasize the possibility of some still unknown type of transmitter in these nerves Whether or not the non adrenergic nerves found are cholinergic the axo axonal interactions at the peripheral neuroeffector level seem to be possible also in the rabbit myometrium

The sex steroids are known to represent the humoral part of the complex neuro-humoral regulation of the myometrial activity Estrogens and progestins are known to act also on the peripheral end of the myometrial innervation (cf Marshall 1970) The changes in the axo axonal relations in the terminal nerve fibres produced by the sex steroids might serve as a physiological mechanism inducing changed myometrial response to nerve stimulation However the importance of the axo-axonal interactions in general remains still to be established as also its importance considering the regulation of the myometrial function

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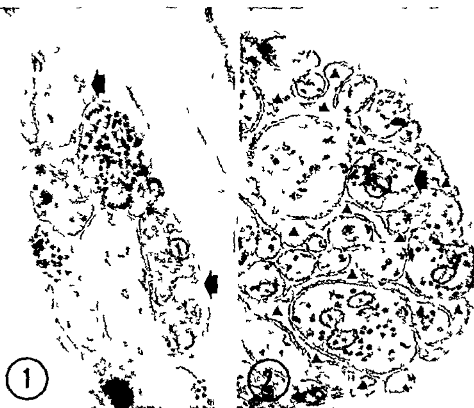


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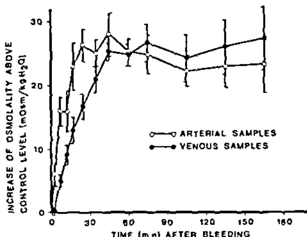
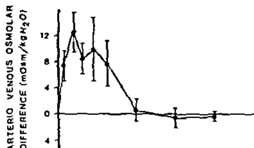


Fig 1 Changes of arterial and femoral (or popliteal) venous osmolality in cats (mean values ± S E M n = 17) after hemorrhage which reduced arterial pressure to 50 mm Hg



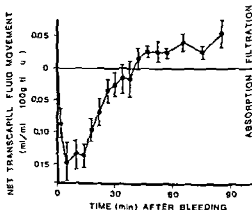


Fig 2 Arterio-venous osmolar differences and net transcapillary flu d fluxes in cat skeletal muscle after hemorrhage wh ch reduced arterial pressure to 50 mm Hg (mean values ± S E M $n = 9$) Influence of sympatho-adrenal system eliminated by reg onal sympathectomy and α adrenergic blockade

appeared The average cumulative fluid absorption during the first 45 min after bleeding was 2 8 ± 0 52 ml/100 g muscle in these experiments Sham-operated normotensive animals (n = 3) showed no significant change of plasma osmolality and no net transcapillary fluid movement

Comments

Hyperglycemia after hemorrhage was first described by Claude Bernard and his finding has been repeatedly confirmed There are some occasional reports on plasma hyperosmolality after bleeding (*e g* Bergentz and Brief 1965 Boyd and Mansberger 1968) but there seems to be no study aimed at revealing its possible role in plasma volume restoration This preliminary investigation performed under circumstances when reflex net transcapillary fluid movements were eliminated indicates that considerable osmotic fluid absorption from the extravascular space (25—30 ml/kg muscle tissue) can result from hyperglycemia induced arterial hyperosmolality in early (< 1 h) stages of hemorrhage It might be argued that the hypotension *per se* could lead to a passive decrease of capillary pressure and hence partly explain the observed fluid absorption Capillary pressure however is not significantly altered even by large reductions of arterial pressure apparently due to efficient autoregulatory mechanisms (*e g* Öberg 1964) This is supported by the present finding that in later stages of hemorrhage when the arterio-venous osmolar difference had vanished there was no fluid absorption despite maintained low perfusion pressure (Fig 2)

Recent findings (Lundvall 1972) indicate that transcapillary osmotic fluid absorption is a blood flow limited process Normal reflex blood flow reduction in response to hemorrhage was prevented in the present experiments and therefore the quantitative role of arterial hyperosmolality in the plasma volume control during bleeding might have been somewhat overestimated This problem is approached in studies in progress

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Studies on Left Ventricular Receptors, Signalling in Non-Medullated Vagal Afferents

By

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Abstract

ÖBERG B and P THORÉN *Studies on left ventricular receptors signalling in non medullated vagal afferents* Acta physiol scand 1972 *85* 145—163

Left ventricular receptors signalling in non medullated vagal fibres and displaying a low irregular spontaneous activity have been analysed by recordings of impulse activity in cardiac afferent nerves The receptors were distributed diffusely all over the ventricle and in both superficial and deep layers of the myocardium They were activated by occlusions of the ascending aorta by substantial elevations of arterial blood pressure and by rapid infusions of saline or dextran whenever these procedures produced a clearcut increase of intraventricular diastolic pressure and end-diastolic volume The endings were also excited by veratrum alkaloids nicotine and digitalis alkaloids When activated the receptors usually displayed a cardiac rhytm but with intense stimulation the activity usually became continuous The receptors evidently function as mechano- or deformation receptors responding primarily to a distension of the ventricle Activation of the receptors causes a reflex bradycardia and in all probability vasodilatation and blood pressure fall They may contribute to the homeostatic control of blood pressure and blood volume but their main function seems to be to protect the heart from *e g* imminent overloading The endings are probably of main importance for the emergence of the Bezold Jarisch reflex

The presence of receptors in the heart ventricles is well documented from both morphological studies (*cf* Hirsch *et al* 1964) and from recordings of impulse activity in afferent fibres from the heart (*cf* Paintal 1963 Sleight and Widdicombe 1965) To judge from such analyses of discharge characteristics in the afferent nerves there seem to exist in principle two main types of ventricular endings One group of receptors fire in medullated afferents with a short burst of activity during the isometric contraction of the ventricles (Paintal 1955) and may be designed as ventricular pressure receptors The second type of endings fire in non medullated afferents and show a low and irregular spontaneous activity (Coleridge Coleridge and Kidd 1964 Sleight and Widdicombe 1965)

A recent study (Öberg and Thorén 1972 a) showed that one type of ventricular receptors with non medullated afferents suddenly increased their activity when the central blood volume was critically reduced by means of rapid blood withdrawal or peripheral pooling of blood This increased receptor discharge was usually accom

panied by a reflex slowing of the heart — In the present study these ventricular receptors have been studied in more detail with regard to their functional characteristics A preliminary report of this study has been published earlier (Öberg and Thoren 1971)

Methods

Cats anesthetized with chloralose 30—50 mg/kg b wt. were used The animals were placed on positive pressure artifical ventilation and the thorax was opened by a right intercostal incision The pericardium was split and the edges suspended to the thorax wall by means of ligatures — Snares were placed around the ascending aorta the pulmonary artery and both caval veins close to their entrance in the right atrium — To get access to the right cardiac nerve running from the heart to the vagus stem in the chest the right upper lung lobe was removed and the azygos vein sectioned between ligatures The cardiac nerve was carefully freed and placed on a bipolar stimulation electrode but otherwise left intact A small nerve filament running from the lung root to the cardiac nerve along the lung vessel was cut if possible without too extensive dissection — The right vagus nerve was also cut in the chest just below the entrance of the cardiac nerve The carotid arteries the vagus nerve the sympathetic trunk and the depressor nerve were dissected free bilaterally in the neck for 1—2 cm The sheath surrounding the vagus nerve on the right side was divided and thin filaments from the nerve dissected free and placed on an electrode for recordings of impulse activity on a double beam oscilloscope together with ECG The impulse activity was also followed by means of a spike counter device connected to a Grass Polygraph Model 7 A recorder — For further details concerning the recording techniques see Öberg and Thoren (1972 a)

Arterial blood pressure was measured by means of a Statham pressure transducer (Model P23AC) and an indwelling catheter in one brachial artery and monitored on a Grass Polygraph recorder Left intraventricular pressure was similarly recorded via a catheter advanced into the ventricular cavity from one carotid artery Heart rate was measured by a tachograph triggered by the systolic uprise of the blood pressure wave — In some experiments ventricular volume changes (both ventricles) were recorded by placing the ventricles in a cardiometer The cardiometer was made essentially airtight by means of a rubber membrane embracing the heart in the atrioventricular plane — Volume changes were monitored on the Grass recorder via a Grass volume transducer (Modell PT 5 A) — One femoral artery was cannulated for bleeding and transfusion purposes — Intravenous injections were made through a catheter in one brachial vein The arterial blood pressure curve ECG the nerve activity and a signal from the Grass Polygraph was fed into a four channel FM tape recorder (Philips Model Ana Log 7) for storage of the data When according to the spike counter there was a change of nerve activity this could be played back from the tape on the oscilloscope and photographed

Conduction velocities in the studied filaments were recorded by applying an electrical stimulation on the cardiac nerve as close to the heart as possible and record he evoked potentials on the oscilloscope The stimulation served as a trigger for the sweep The in situ length between recording and stimulation electrodes were measured as carefully as possible

Experimental procedure To facilitate the identification of ventricular receptor afferents in the right cervical vagus during the dissection the aorta was repeatedly occluded for a few seconds By this manoeuvre the discharge of ventricular receptors increased markedly If during the dissection a vagal filament was found showing an increased activity during aortic occlusion the dissection continued until one or only a few active fibres remained in the preparation which could often be separated because of differences in spike configuration Sometimes however records were made from multifibre filaments where the individual afferents could not be identified with certainty

Results

This study is based on 22 successful experiments with recordings of impulse activity in 50 afferent filaments from receptors which were localized to the left ventricle by their response to aortic occlusion and mechanical stimulation of the heart The 50 filaments include 14 single fibre preparations 25 multifibre preparations containing 2—3 active afferents and 11 filaments with more than 3 active fibres

Spontaneous activity The receptors showed a very low spontaneous activity with a discharge rate varying between 0 and 5 imp/s (mean 1 0 imp/s) About one third of the receptors were normally silent Less than 20 % of the spontaneously active receptors displayed a cardiac rhythm while the remaining fired irregularly without any fixed relation to the QRS complex of the ECG Taking the conduction time from the heart to the recording site into account the rhythmic discharge was estimated to occur usually in diastole, but some receptors seemed to be activated during systole or during both systole and diastole The exact firing position in the cardiac cycle was however difficult to establish because of uncertainties with regard to the total intrathoracic conduction time

Conduction velocities and spike morphology Conduction velocities were measured in 8 single fibre preparations and in 14 multi fibre filaments containing 2 or 3 active afferents The conduction velocities varied between 0 4—3 5 m/s (mean 0 9 m/s) Only 2 afferents had conduction velocities exceeding 2 m/s (2 5 and 3.5 m/s respectively) One of these 2 receptors differed from all the others also in that respect that it was extremely sensitive to a very light touch of the heart and was therefore probably located very superficially possibly in the epicardium —The action potentials appeared as long lasting spikes with a duration of 1 5 ms or more They could also be identified in the loudspeaker by the less distinct and low pitched sound compared with that resulting from activity in myelinated fibres from *e g* atrial receptors —The recorded spike amplitudes varied between 10 μV (around noise level) up to 80 μV

Receptor response to aortic occlusion The studied filaments were as mentioned selected according to their increased activity during short lasting occlusion of the ascending aorta The disharge started to inrease within 1—2 s after the onset of the occlusion and often initially displayed a clear cardiac rhythm with a burst of impulses after the QRS complex in ECG This means if the conduction time from the heart to the recording site (150—250 ms) is taken into account that the receptors were activated during diastole Towards the end of the occlusion period the receptor activity usually became continuous with a maximal impulse frequency varying between 3 and 25 imp/s—Only occasionally a tendency for adaptation was seen during the short lasting (5—10 s) occlusion

Simultaneous recordings of afferent nerve activity and left ventricular pressure during graded aortic occlusion were made in 18 filaments Fig 1 shows recordings from one such experiment The activity is recorded from a filament containing afferents from two receptors They show during control conditions a low irregular activity With a moderate obstruction of the aorta leading to a rise of the intraventricular systolic pressure (LVSP) while the ventricular diastolic pressure (LVDP) remained unchanged there is an essentially unchanged receptor activity (neurogram A) When the aorta is more severely constricted so that also LVDP starts to rise there is a sudden marked increase of receptor activity which initially displays a cardiac rhythm (neurogram B) but later becomes continuous (neurogram C)

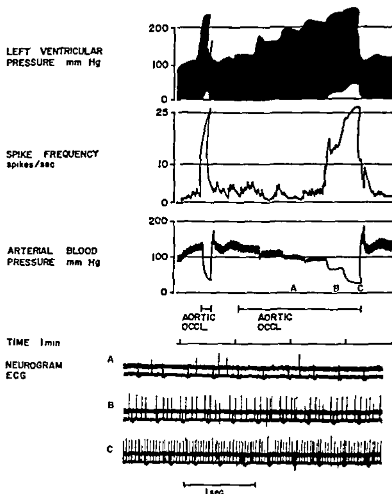


Fig 1 Effects of progressive obstruction of the ascending aorta on left intraventricular pressure arterial blood pressure and spike frequency in two non myelinated cardiac afferent fibres The neurograms in this and subsequent figures are recorded at times denoted by respective letter An increased receptor activity is obtained first when the intraventricular diastolic pressure is significantly elevated The receptors then fire with a cardiac rhythm (neurogram B) With a complete aortic occlusion the receptors fire continuously (neurogram C)

This pattern of receptor response was typical for all examined receptors They thus increased their activity first when a clearcut rise of LVDP was observed A partial occlusion of the aorta caused in many cases only a short lasting increase of LVDP which was then regularly accompanied by a transient increase in receptor discharge

The individual receptors showed fairly marked variations with regard to threshold Thus some receptors that were normally silent started to fire when LVDP was just discernably raised while others were not activated until LVDP was elevated above 20 mm Hg and fired then with only one spike per heart cycle

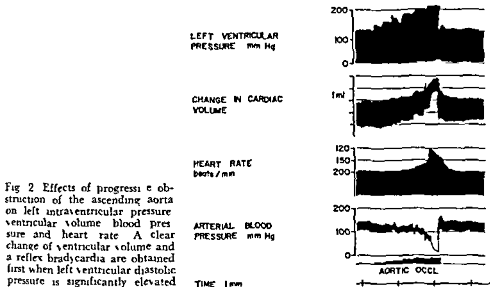


Fig 2 Effects of progressive obstruction of the ascending aorta on left intraventricular pressure ventricular volume blood pressure and heart rate A clear change of ventricular volume and a reflex bradycardia are obtained first when left ventricular diastolic pressure is significantly elevated

In 3 expts ventricular volume changes were followed during progressive obstruction of the aorta Records from one such experiment is shown in Fig 2 With a moderate constriction of the aorta there is a marked increase of LVSP but no evident change in LVDP or cardiac volume First with a more severe obstruction there is also a rise of LVDP accompanied by a clear increase of end systolic and end diastolic volumes Simultaneously a bradycardia develops which on further analysis was found to be of reflex origin since it disappeared with cooling of the cervical vagi —The described effects are further augmented when the aorta is totally occluded —It thus seems that activation of the receptors during severe aortic occlusion is related to a distension of the ventricles and that the receptors then initiate a reflex bradycardia

Receptor responses to intravenous adrenaline and carotid occlusion In a series of experiment the receptor activity was studied when the outflow resistance from the left ventricle was increased in a more natural way by elevating the arterial blood pressure by means of adrenaline injections or carotid occlusions 9 of 13 tested filaments showed a markedly augmented activity when adrenaline was injected i v but only when the adrenaline induced rise of the arterial pressure was so pronounced that LVDP also increased Fig 3 illustrates this phenomenon The studied filament contains afferents from 3 left ventricular receptors of which only one is spontaneously active (A) With the lowest dose of adrenaline there is no change of receptor activity With 5 μg the blood pressure rise is accompanied by a barely dicernable elevation of LVDP and then there is also a slight increase of receptor activity (B) With 10 μg adrenaline there is a clearcut increase of LVDP and receptor activity and the two previously silent receptors also became excited (C) —The receptor

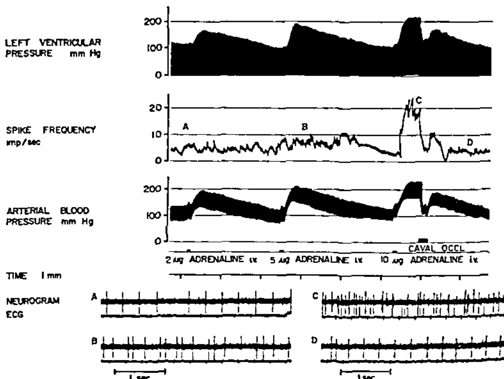


Fig 3 Effects of intravenous injections of adrenaline on blood pressure left ventricular pressure and spike frequency in a filament containing afferents from 3 ventricular receptors Note that only one receptor is spontaneously active This receptor shows an increased activity when the blood pressure is moderately elevated causing a barely noticable rise of the ventricular diastolic pressure (B) With more marked blood pressure responses so that the left ventricular diastolic pressure becomes significantly elevated all three receptors become activated (C) Normalization of blood pressure by means of a partial occlusion of the caval veins is seen to bring back impulse activity to normal

activation is evidently related to the rise of LVDP since when this pressure is brought back to control during maximal adrenaline influence by partial occlusion of the caval veins receptor activity also declines abruptly (Fig 3) However one got the impression that when the arterial blood pressure was elevated to the same extent either by adrenaline or by aortic occlusion the receptors discharged more heavily during the influence of adrenaline than during constriction of the aorta This may indicate that adrenaline activates the receptors not only by way of a distension of the ventricles caused by increased arterial blood pressure and LVDP but also by way of the effects on ventricular contractility

In a number of experiments the carotid arteries were occluded If a marked pressor response was then obtained there was also a rise of LVDP and an increased receptor activity as illustrated in Fig 4 This filament contains 2 afferents which can be separated by the different spike configuration The fibre with the smaller spike is spontaneously active the other fibre with a pronounced downward deflec-

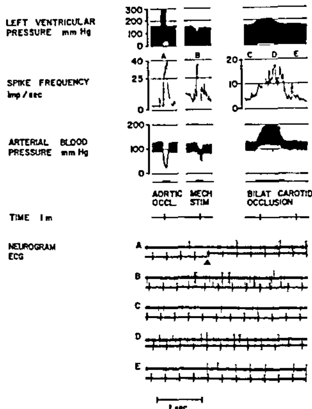


Fig 4 Effects of aortic occlusion mechanical stimulation of the anterolateral part of the left ventricle and bilateral carotid occlusion on left intraventricular pressure blood pressure and impulse activity in a vagal filament containing afferents from 2 ventricular receptors Only one of them shows a low spontaneous activity (left part of neurogram A) but both are strongly activated by aortic occlusion (A) mechanical stimulation (B) and carotid occlusion (D) Note the rise of left ventricular diastolic pressure with the latter procedure

tion is essentially silent but becomes activated by aortic occlusion (A) and mechanical stimulation of the heart (B) When both carotid arteries are occluded LVDP is seen to rise and simultaneously there is an increased activity in the spontaneously active fibre and the previously silent receptors became activated (D)

Receptor responses to expansion of the blood volume To further analyse the possible relation between ventricular end-diastolic volume and receptor discharge a series of experiments were performed in which the ventricles were distended by rapid injections of saline or dextran (8—10 ml in 3—5 s) intraventricularly With such injections 3 of 5 tested filaments increased their impulse activity (from in average 1 to 6 imp/s) Fig 5 shows records from one experiment where a filament containing 2 afferents was analysed Under control conditions only one receptor is spontaneously active with a low irregular discharge (neurogram A) A rapid injection of saline into the ventricle leads to an instantaneous increase of receptor discharge initially with a clear cardiac rhythm (B) which with further receptor activation becomes less evident (C) The previously silent receptor also starts to fire with a low irregular impul e rate —The receptor activity returns slowly towards normal and remains somewhat elevated as compared with the control situation for one to two minutes

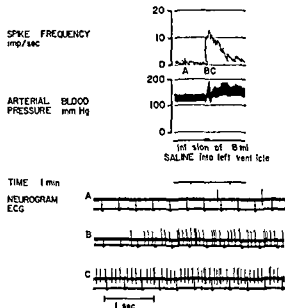


Fig 5 Effects of rapid saline infusion into the left ventricle on impulse activity in a vagal filament containing afferents from two receptors located in the apex region of the left ventricle Note the very low spontaneous activity and that the receptor shows initially a cardiac rhythm when activated

The activity in 3 filaments was examined when intravascular volume was expanded in a stepwise fashion by repeated injections of 5 ml saline intraarterially Fig 6 shows recordings from a multi fibre filament containing afferents from 3 receptors of which only one is spontaneously active (neurogram A) Injection of 5 ml saline i a causes only a transient increase in LVDP and receptor discharge When a total of 10—15 ml saline has been administered there is a sustained slight elevation of LVDP and receptor activity (B) which is further augmented by additional transfusion (C) In this situation all three receptors are active and fire with a cardiac rhythm —Withdrawal of 15 ml blood brings the normal situation back to normal (D) —The remaining two fibres studied in this series required more pronounced volume alterations to increase their activity but showed otherwise essentially the same response pattern

Receptor responses to occlusion of the pulmonary artery The receptor response to occlusion of the pulmonary artery for about 20 s was tested in 35 filaments 7 filaments (2 single fibres) showed an instantaneous rapid increase of activity within 2 s after the onset of occlusion In 18 filaments there was after a latency a gradual moderate increase of activity which occasionally displayed a cardiac rhythm This later type of receptor response thus closely resembled that seen during rapid severe bleeding of the animal and during occlusion of the caval veins and was therefore apparently due to a depletion of the left ventricle and the consequent squeezing of the myocardium (Öberg and Thorén 1972 a) —In 10 filaments no alterations of activity were observed during occlusion of the pulmonary artery

Receptor responses to alterations of lung volume Collapse or overinflation of the lungs had no effects on receptor activity in 5 studied filaments

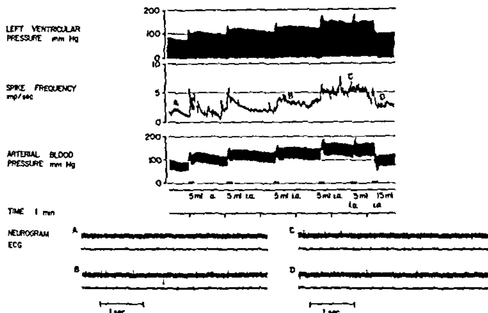


Fig 6 Effects of repeated injections of saline intraarterially on blood pressure left ventricular pressure and spike frequency in a filament containing afferents from 3 ventricular receptors Only one receptor is spontaneously active but with repeated injections all three receptors became activated and display a cardiac rhythm (C) The progressive diminution of spike amplitude is due to a deterioration of the filament.

Receptor responses to mechanical stimulation of the heart location of the receptors The described receptors were activated when the exposed epicardial surface of the heart was mechanically stimulated Only one of the 35 studied filaments responded to an extremely light touch of a localized area at the lateral part of the left ventricle and was therefore considered to be very superficially located probably in the epicardium The remaining 34 filaments required a more firm stroking or pressure or even squeezing and massage of the left ventricle to become activated Such explorations indicated that the receptors were diffusely distributed in all parts of the left ventricle which could be adequately examined Most receptors were found to be located in the antero-lateral wall but many endings were also found in the diaphragmatic part of the ventricle The precise location of receptors which responded only to a firm squeezing or massage of the ventricle could of course not be determined Of the receptors showing an instantaneous rise of activity with occlusion of the pulmonary artery two were activated by firm stroking over the heart at the interventricular groove —None of the examined receptors was stimulated by mechanical probing restricted to the right ventricle only

Receptor responses to various drugs a) Nicotine Local application of nicotine (50 µg) in the pericardial cavity was found to excite approximately one third of

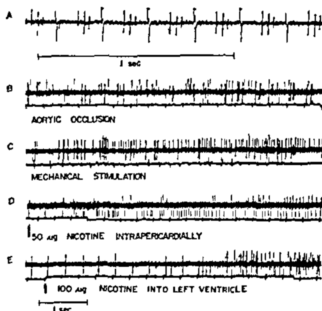


Fig 7 Effects of aortic occlusion mechanical stimulation and nicotine administered intrapericardially or intraventricularly on impulse activity in a vagal filament containing afferents from 3 receptors Neurogram A shows the evoked potentials when the right cardiac nerve is stimulated Conduction velocities of the three afferents were estimated to 0 8 0 6 and 0 5 m/s respectively Only one receptor is spontaneously active (e g left part of F) All 3 receptors are activated by aortic occlusion (B) and nicotine injected into the left ventricle (E) *while two receptors respond to* firm pressure over the apex region (C) Intrapericardial injections of nicotine excites only one receptor (D)

the tested receptors Maximal discharge rate was around 25 imp/s The increased receptor discharge appeared with a latency of 0 3—12 s in 70 % of the cases the latency was below 2 s These discrepancies with regard to latency and sensitivity to nicotine are in all probability related to different receptor positions at various depths the wall and not a manifestation of two functionally different receptor populations This idea was supported by the finding that the receptors which responded to intrapericardial administration of nicotine were also excised by a fairly moderate mechanical stroking of the epicardial surface which suggests a rather superficial location in the ventricular wall The receptors which did not respond to nicotine intrapericardially usually required a firm pressure or even squeezing of the left ventricle to become excited which seem to indicate that they were positioned in deep wall layers —Such deeply located receptors could however be activated by nicotine *administered intravenously (1 expt) or intraventricularly (4 expts) in large doses* (40—100 µg) Fig 7 illustrated this phenomenon In neurogram A the evoked potentials were recorded for determinations of the conduction velocities This record demonstrates that the filament contains 3 fibres of which only one is spontaneously active (e g left part of neurogram E) With aortic occlusion (B) all three receptors are activated while firm pressure over the apex region (C) excited only the 2 receptors which discharged in the afferents with the large spike amplitudes Injection of 50 µg nicotine intrapericardially (D) leads to activation of only *one* receptor but when 100 µg nicotine is injected into the left ventricle (E) all 3 receptors are activated after a certain latency

b) Veratrum alkaloids Receptor responses to intravenous injections of protoveratrine in doses of 2– 10 µg were tested on 6 filaments They all increased their

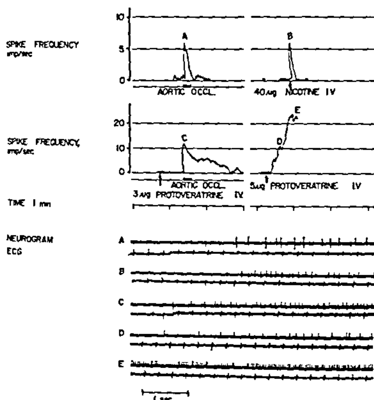


Fig 8 Effects of aortic occlusion intravenous injections of nicotine and protoveratrine on impulse activity in a single fibre filament with practically no spontaneous activity The receptor responds to aortic occlusion (A) and nicotine (B) No altered receptor activity is seen after 3 µg protoveratrine but the receptor response to aortic occlusion becomes more pronounced and prolonged (C) (Note the change of the scale for spike frequency) A larger dose of protoveratrine (5 µg) causes the receptor to fire heavily (D and E)

activity although the threshold dose for the individual endings varied somewhat In two experiments it was found that the receptors were not activated by small amounts of protoveratrine (2—3 µg) but they evidently became sentitized by that dose because their response to aortic occlusion was then markedly augmented and above all prolonged This is illustrated in Fig 8 in which experiment a single receptor afferent with practically no spontaneous activity was analysed The receptor responses to aortic occlusion and nicotine intravenously are shown in neurogram A and B Injection of 3 µg protoveratrine did not in itself excite the receptor but the response to aortic occlusion is now more pronounced (note altered sensitivity for the spike counter!) and prolonged (C) Additional injection of 5 µg protoveratrine 10 min later in the experiment caused the receptor to discharge heavily initially with a cardiac rhythm (D) but later continuously (E)

c) Strophantine I v injections of strophantine in doses of 60—120 µg were tested in 4 filaments Single injections of 60—100 µg strophantine did not affect the spon

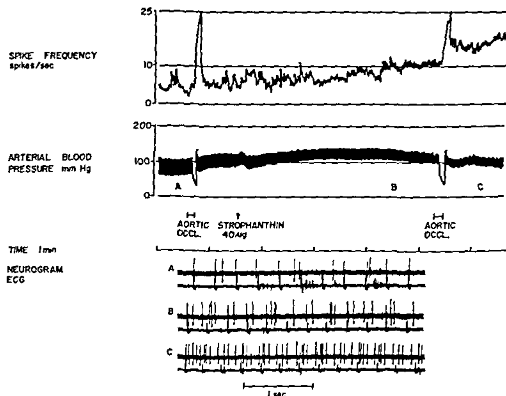


Fig 9 Effects of strophantine on blood pressure and impulse activity in a vagal filament containing afferents form 2 receptors (A) 60 μg strophantine was given 1 h earlier in the experiment An additional injection of 40 μg of the drug causes a progressive increase of receptor activity (B) and a prolonged receptor response to aortic occlusion (C)

taneous receptor discharge but seemed to cause a sensitization of the receptors in the same way as protovertarine did Aortic occlusion performed after injection of strophantine was thus found to produce a more pronounced receptor discharge than before administration of the drug Repeated injections of strophantine to a total amount of 100 μg or more caused an increase also of the spontaneous activity in two tested filaments One of these is shown in Fig 9 This filament contains afferents from 2 receptors with a low spontaneous activity and a hinted cardiac rhythm (A) In this experiment 60 μg strophantine had been given approximally one hour earlier in the experiment The receptors show the usual rapid rise and decline of activity with onset and release of aortic occlusion With an additional injection of 40 μg strophantine bringing up the total amount of drug administered to 100 μg there is after certain latency a progressive slow increase of receptor discharge (B) This increased spontaneous activity lasted for the rest of the experiment

d) Histamine Histamine in large doses (100 μg) did not affect the activity in 2 tested filaments

Discussion

In the present study, the functional characteristics of a group of left ventricular receptors have been analysed by recordings of the afferent impulse activity in non medullated vagal afferents For technical reasons the recordings were made from filaments in the right vagus nerve but it is probable that many or perhaps even most receptors of the described type have their afferent connections in the left vagus nerve The receptors usually showed a low and irregular spontaneous activity Upon activation however they exhibited a rhythmic discharge with a train of impulses which in most cases evidently occurred in diastole When receptor excitation was intense however they discharged continuously throughout the whole cardiac cycle

The receptors were stimulated whenever the ventricles became distended either by an augmented diastolic filling or by an increase of the outflow resistance to such an extent that the left ventricular diastolic pressure became elevated Very forceful contractions of the ventricle as under strong sympathetic influence also seem capable to excite the receptors particularly in situations with extremely low residual blood volume (Öberg and Thoren 1972 a) The receptors can therefore be classified as mechanoreceptors responding to a deformation or distortion of the ventricular wall —Asphyxiation of the animal did not affect the receptor discharge provided the asphyxia period was not so prolonged that secondary severe disturbances of cardiac dynamics occurred (Thoren 1972)

The receptors were also excited by veratrum alkaloids and by nicotine Approximately one third of the receptors responded when nicotine was administered intrapericardially and was therefore supposedly located in superficial wall layers This assumption is supported by the observation that the same receptors were also activated by a moderately strong probing of the heart —The endings which were not activated by nicotine placed in the pericardial cavity did however respond when the drug was injected intravenously or directly into the ventricular cavity in high doses (40—100 μg) Since the same receptors required a more powerful probing of the heart to become activated they were in all probability positioned in deeper layers of the myocardium It therefore seems as if the presently described receptors are distributed all through the thickness of the ventricular wall and that all of them are sensitive to nicotine although depending on their position different administration routes must be employed

According to the receptor responses to mechanical stimulation of the epicardial surface of the heart they seem to be located diffusely all over the left ventricle possibly also in the intraventricular septum None of the receptors showed an increased activity when the mechanical stimulations were restricted to the right ventricular wall It is possible however that a more systematic exploration *e g* by selecting the filaments for study according to the increased activity following pulmonary artery occlusion will reveal the presence of at least a few receptors of the present type also in the right ventricle

Receptors resembling the present ones were described by Jansch and Zotterman (1948) who demonstrated an increased activity in vagal filaments with slowly con

ducted, small spikes most likely non medullated C fibres with pinching of the left ventricle, injections of veratrum alkaloids and occlusion of the aorta Ventricular receptors showing a low irregular spontaneous activity, signalling in C fibres have also been described by Coleridge, Coleridge and Kidd (1964) and by Sleight and Widdicombe (1965) in dogs According to the extent of receptor excitation caused by intrapericardial application of nicotine Sleight and Widdicombe separated the receptors in two groups nicotine sensitive and nicotine insensitive endings which behaved somewhat differently also in other respects The nicotine insensitive receptors were among other things relatively insensitive to aortic occlusion but responded more often to a very light touch of the heart indicating a very superficial possibly epicardial location The nicotine sensitive receptors on the other hand were less sensitive to mechanical stimulation but were prone to respond to aortic occlusion They were therefore probably more deeply located in the myocardium The present receptors resemble the nicotine sensitive receptors of Sleight and Widdicombe although they differ in that respect that most of them were not excited by nicotine intrapericardially but did respond to intravenous administration of the drug This discrepancy can in all probability be explained by the fact that different methods were employed to identify and select the receptors for analysis in the two studies and that therefore the endings are positioned at somewhat different depths of the myocardium Sleight and Widdicombe thus selected and studied receptors characterized by their increased activity when the epicardial surface of the heart was palpated and their nicotine sensitive endings therefore probably constitute a rather superficially located population of myocardial receptors easily reached by nicotine placed in the dial cavity The receptors presently studied were on the other hand selected according to their increased discharge when the aorta was briefly occluded and include therefore probably also more deeply located receptor endings out of reach for nicotine diffusion from the epicardial surface In the present explorations only one receptor was found which resembled the nicotine insensitive endings of Sleight and Widdicombe This rare occurrence of very superficially located receptors in the present study might reflect a species difference but is again most likely related to the method presently used to select receptors for study

No attempts were presently made to analyse systematically the *circulatory reflex effects* produced by activation of the ventricular receptors There was evidence however that a reflex bradycardia of vagal origin often accompanied an increased receptor activity Since various procedures which according to the present study activate the receptors such as pronounced pressure elevations in and consequent distension of the left ventricle (*e g* Aviado and Schmidt 1955 1959 Salisbury Cross and Rieben 1960) intravenous injections of veratrum alkaloids (*e g* Dawes and Comroe 1954) and intrapericardial administration of nicotine (Sleight 1964) induce a blood pressure fall bradycardia and a peripheral vasodilatation and since electrical stimulation of non medullated afferents in the cardiac nerves produce similar effects (*e g* Öberg and White 1970 Öberg and Thorén 1972 b) it is reasonable to assume that the present receptors exert a generalized inhibitory influence on the bulbar

vasomotor centre This generalized depressor function of the receptors may however imply a particularly strong activation of vagal efferents to the heart and inhibition of the vasomotor neurons to the kidney since this is known to occur when non medullated cardiac afferents are electrically stimulated (Öberg and White 1970)

The functional significance of the present receptors and their reflex effects on the circulatory system is uncertain The observation that the receptors are strongly activated when the blood pressure is markedly elevated seems to indicate that they in such extreme situations work in consent with the arterial baroreceptors to bring the pressure back to normal levels This agrees with the findings that interruption of the impulse traffic from cardiac receptors by cutting or cooling the vagi or the cardiac nerves leads to marked pressor responses in case the blood pressure is initially markedly elevated by *e g* elimination of arterial baroreceptor restraint (Pillsbury Guazzi and Freis 1969 Öberg and White 1970) and that blood pressure responses to carotid occlusion become augmented after section of the cervical or intrathoracic vagi (Guazzi Libretti and Zanchetti 1962 Öberg and White 1970) The low and often absent spontaneous activity of the receptors gives however, the impression that they under normal conditions exert only a moderate tonic inhibitory influence on the circulation It should be remembered however that the present experiments were performed on animals with opened thorax and therefore with small cardiac volumes (Rushmer Finlaysson and Nash 1954) Considering the dependence of receptor activity on the prevailing ventricular volume as evident from the present results this might imply that the spontaneous receptor activity is abnormally low in thoracotomized animals and that a more pronounced tonic activity prevails in intact closed chest preparations The findings (Öberg and White 1970) that the pressor responses to vagal cooling became markedly attenuated by thoractotomy but again restored after expansion of the blood volume supports this view —If so the present receptors may respond to both elevation and reductions of blood pressure from the normal level and thus act as true buffers contributing to the maintainance of blood pressure homeostasis This blood pressure regulating function of the present receptors is however probably of little importance in comparison with the arterial baroreceptors since in the presence of normally operating baroreceptor reflexes even closed chest preparations show only small blood pressure rises when the cardiac receptor influence on central vasomotor neurons is eliminated (Öberg and White 1970)

The fact that a conspicious increase of receptor activity appeared quite suddenly first when the aortic pressure attained a critical value where the left ventricle became distended rather suggests that the receptors are engaged in protective mechanisms producing marked blood pressure falls in case of imminent overloading of the heart Such a protective function of ventricular receptors was indeed suggested already by Daly and Verney (1927) The fact that a sudden activation of the receptors occurs when the ventricles contract vigourously around an almost depleted chamber as during severe hemorrhage of peripheral pooling of blood (Öberg and

Thorén 1972 a) suggests that they also in such circumstances exert a protective function aiming at preventing a squeezing and tearing of the myocardium The reflexly induced bradycardia and consequent improvement of the diastolic filling of the ventricles seems to be of advantage in this particular situation

The receptor response to an increased blood pressure appeared particularly marked when the contractile force of the ventricle was simultaneously augmented as *e g* with adrenaline injections It is conceivable that the extent of deformation or rate of deformation of the endings is then more intense Such strong excitation of ventricular receptors may explain the findings of Konzett and Rothlin (1951) that intravenous administration of adrenaline in large doses (10—20 μg) is sometimes followed by a marked bradycardia and blood pressure fall provided the vagi are intact

The increase of receptor activity when the ventricular end diastolic volume was increased by rapid infusions of *e g* saline, and the diminution of the spontaneous receptor activity during progressive hemorrhage (Öberg and Thorén 1972 a) suggests that they may act as volume receptors engaged in the homeostatic reflex control of blood volume Such a control is evidently executed mainly by hormonal efferent pathways (Gauer *et al* 1970) but possibly also to a certain extent by alterations of vasoconstrictor fibre outflow to the renal vessels which seem to be particularly strongly influenced from cardiac receptors (Öberg and White 1970) —Some endings were found to respond to relatively small alterations of blood volume and hence of ventricular end diastolic volume and increased their activity markedly when intravascular volume was increased by less than 10 % Even if these endings represent a small low threshold group of the receptor population their reflex influence may be potent enough to induce significant alterations of renal hemodynamics —It should be noted in this context that the pericardium was regularly opened in the present experiment which may allow the heart to attain exceptionally large volumes with expansions of the blood volume The maximal level of receptor activation presently recorded with large transfusions may therefore not occur normally when the pericardium is intact but the general receptor response characteristics to volume changes are probably not significantly influenced

The pronounced reflex bradycardia resulting from activation of the present receptors by rapid transfusions might seem less expedient since it implies a further augmentation of the diastolic filling and of receptor activity Such a positive feed back must reasonably be opposed by counteracting mechanisms The concomitant reflex vasodilatation evidently affecting also the veins (Ross Frahm and Braunwald 1961 Öberg and White 1970) with a tendency to peripheral blood pooling and decreased venous return may constitute such an opposing mechanism Another counteracting factor may arise from atrial receptors excited by the simultaneous distension of the atria and inducing a reflex acceleration of the heart (Ledsome and Linden 1964) It seems probable however that this excitatory influence on the heart of atrial receptor origin is often overpowered by the strong inhibitory influence from ventricular receptors and that therefore a reflex tachycardia or a Bainbridge

is only occasionally observed with rapid transfusions into the atria

Furthermore it is possible that the reflex decrease of activity in sympathetic fibres to the heart reported by Hakumaki (1970) to occur with transfusions into the left atrium and by him ascribed to atrial receptor activation is rather due to a simultaneous excitation of ventricular receptors of the presently described type

The importance of ventricular non medullated endings in producing the so-called Bezold reflex has been pointed out by previous workers (e g Jarish and Zotterman 1948 Sleight and Widdicombe 1965) and is also evident from the present experiments Even if veratrum alkaloids are capable to excite also receptors with medullated afferents such as atrial receptors (Paintal 1957) and ventricular pressure receptors (Paintal 1955 Brown 1965) the present receptors seem to be activated by smaller doses of the drug—The present endings are apparently also numerous enough to exert a powerful restraint on the vasomotor centre while *e g* the ventricular pressure receptors seem to be very sparse as pointed out by *e g* Coleridge *et al* (1964) In fact not a single ventricular pressure receptor was found in the present study despite extensive explorations Furthermore the present receptors seem to be preferentially located in the left ventricle *i e* in that part of the heart from which the Bezold reflex is said to be elicited at least in dogs (*e g* Dawes 1947) The ventricular pressure receptors on the other hand seem to be fairly equally distributed in both ventricles (Paintal 1955) Finally the circulatory adjustments in the Bezold reflex can be mimicked by afferent stimulation of the cardiac nerves only when such stimulation intensities are used which activate non medullated afferents For such reasons it seems as if receptors of the present type are of main importance for the emergence of the Bezold reflex

The bradycardia obtained with administration of digitalis is known to be partly due to an increased efferent vagal activity (*cf* Chai *et al* 1967) The present experiments indicate that one important mode of action of digitalis is to activate ventricular receptors and to induce a Bezold reflex Injections of digitalis alkaloid in large doses are indeed said to induce a Bezold response (Fukuda 1950 and Melville 1952) which is not surprising considering the similar basic steroid structure of digitalis and veratrum alkaloids (Krayer and Acheson 1946) —In the present study fairly large doses of strophantine were required to sensitize or activate the receptors The exact threshold dose could however not be estimated since maximal effects of the drug are obtained first after 1/2—2 h while the thin nerve filaments recorded from in the present study seldom maintained a normal function for more than 30 min A gradually developing excitation of receptors may therefore occur with considerably lower doses of the drug The tendency for a sensitization of the receptors with strophantine is interesting because if this occurs with therapeutic doses of digitalis the consequent reflex bradycardia should be particularly pronounced in diseased dilated hearts where the receptor activity is presumably initially high than in hearts of normal sizes

Johnsson (1971) recently propo ed that some characteristic features of severe aortic stenosis such as bradycardia hypotensive syncope and ventricular fibrillation

may constitute reflex effects of an increased activity in cardiac receptors excited by the elevated pressure in the ventricle The present receptors may play an important role in this situation since they are strongly activated by aortic occlusion and are then capable to induce bradycardia and possibly such a pronounced fall of blood pressure that cerebral and coronary blood flows become inadequate

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Increased Activity in Left Ventricular Receptors during Hemorrhage or Occlusion of Caval Veins in the Cat — A Possible Cause of the Vaso-vagal Reaction

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Abstract

ÖBERG B and P THOREN *Increased Activity in Left Ventricular Receptors During Hemorrhage or Occlusion of Caval Veins in the Cat — A Possible Cause of the Vaso vagal Reaction* Acta physiol scand 1972 85 164—173

Impulse activity in vagal afferent fibres and changes in heart rate were simultaneously recorded during rapid hemorrhage or blood pooling in cats in order to elucidate whether the sudden reflex bradycardia sometimes evoked with the mentioned interventions was correlated to an increased activity in any type of heart receptors The results show that the slowing of the heart resulting from an emptying of the central blood reservoirs was correlated to and eceded by an increased activity in receptors located in the left ventricle and signalling in non medullated afferents The receptors were found to be activated also by obstructions of the ascending aorta and mechanical stimulation of the heart and therefore seem to operate as mechanoreceptors stimulated by a distortion of the myocardium — It is conceivable that with rapid bleeding or pooling of blood the receptors are excited by an improper squeezing of the myocardium when the ventricles contract vigorously around an almost empty chamber and then induce a reflex bradycardia. This reflex mechanism resembling the so-called vaso-vagal syncope *reaction in man may therefore serve as a protective system* causing a break on the heart and allowing for an improved diastolic filling in situations when venous return is critically reduced

In a recent study it was shown (Öberg and White 1970) that a rapid and severe hemorrhage in cats sometimes produced a reflex bradycardia similar to that seen in the so called vaso vagal syncope reaction in man This reflex slowing of the heart was found to be triggered from receptors with their afferent pathways in the card ac nerves because section of these nerves eliminated the response This interruption of the afferent pathways from the receptors did not in itself produce any brady cardia and it was therefore concluded that this reflex response was caused by an activation of some type of receptor during hemorrhage and not to a progressive unloading of tonically active receptors — The precise localization and functional characteristics of these postulated receptors were not further elucidated in the men tioned study

In the present series of experiments impulse activity in vagal afferents from the heart was recorded during hemorrhage or occlusion of the caval veins in order to establish whether the sudden appearance of a reflex bradycardia with these manoeuvres was correlated to an increased activity in any type of receptors and if so to estimate the location of these endings A preliminary report of this study is presented elsewhere (Öberg and Thoren 1970)

Methods

Experiments were performed on cats anesthetized with chloralose 30—50 mg/kg b wt A tracheal cannula was inserted and the animals placed on positive pressure artificial respiration The thorax was opened by an intercostal incision prolonged acr s the midline by di ision of the sternum The pericardium was split and the edges suspended by ligatures from the thorax wall Snares were placed around the ascending aorta and the pulmonary artery so that the pressure could be selective elevated in the left and right side of the heart respectively Snares were also placed around both caval veins close to their entrance in the right atrium so that the central blood volume could be reduced by peripheral pooling of blood The right cardiac nerve running from the heart to the vagal stem in the che t was dissected free after division of the azygos vein and in later experiments removal of the right upper lung lobe A separate branch from the cardiac nerve running with the lung vessels to the lung root was cut in most cats — The vagal stem was likewise cut in the chest below the entrance of the cardiac nerve

The carotid arteries the vagal nerves and the sympathetic trunk were freed and separated from each other bilaterally in the neck The aortic nerves were identified and cut. — The nerve sheet surrounding the right vagus nerve was carefully removed by means of sharp forceps and by cautious dissection thin filaments were obtained and cut centrally The remaining part of the vagal stem was left intact The filament was placed on an electrode for registration of impulse activity For this purpose the ele trode was connected via a preamplifier to one channel of a Tektronic 502 double beam oscilloscope The electrocardiogram was recorded on the other channel — The pre amplifier was equipped with separate filters for high and low frequency cut off High frequency cut off could be changed from 150 kHz to 250 Hz in 6 steps and the low frequency cut off in 5 steps from DC to 100 Hz When recordings were made from medullated fibres the high frequency cut off was set to 1000 Hz but with non medullated fibres it was more convenient to use 250 Hz — The pre amplifier was also connected to a loud speaker and to a rate meter equipped with a discriminator which could select for counting either all spikes with a spike amplitude exceeding a preset value or only spikes with amplitudes between two predetermined levels The spike rate was recorded on a Grass Polygraph recorder Full scale deflection on the recorder could be chang d from 5 to 500 spikes per sec The time constant for the rate meter could be varied bet een 0 4 to 7 s

Arterial blood pressure was measured from one femoral artery by means of a Statham pressure transducer (P 23 AC) and recorded on the Grass recorder Left ventricular pressure was measured in some experiments v a a catheter advanced into the ventricle from one carotid artery Heart rate was monitored by means of a tachograph on the Grass recorder The tachograph was triggered by the systolic upstroke of the arterial blood pressure v a e — A catheter was placed i the othe femo al artery for bleeding purposes — The rectal temperature was checked and kept at 36—38 C by means of external heating

The arterial blood pressure ECG the nerve activity and the signal from the Grass recorder were connected to a four channel FM tape re order (Phil ps Model Ana Log 7) for storage of data When according to the p ke ou ter and the loud speaker alterations of nerve activity occurred as a response to an experimental nterv ntion the nerve activity could be played back on the oscilloscope and photographed

Conduction velocities in afferent fibres were determ ned by applying an electrical stimulation to the exposed but otherwise intact right ardiac nerve and record ng the evoked potentials on the oscilloscope with a high sweep speed The sweep was triggered by the st mulation The potentials were identified on th oscilloscope tracing and the d stance from stimulat on and re ord ng electrodes m asured

E perimental procedure In the e ly experiments d ssection of the right vagus nerve continued until a filament containing one or a fe acti e fibres was obtained The filament was then tested with respe t to acti ty change d ing hemorrhage This t chnique pro ed adequate when atrial receptor afferents were tudi d because the relatively large spike amplitude pronounced spontaneous discharge and charact ristic cardiac rhythm made them easy to

identify. With regard to the ventricular receptors to be described this approach was less profitable since the normally small spike amplitude and low spontaneous discharge without audible cardiac rhythm made it difficult to decide whether the studied filament really contained any active afferent fibre from ventricular receptors. However it was soon evident that the receptors greatly increased their activity when the aorta was occluded for 5—10 s. In subsequent experiments therefore the activity in the dissected filaments were routinely tested with repeated short lasting occlusions of the aorta and in case there was an increased activity the filament was used for further dissection until one or a few active filaments remained. The activity in these filaments was then studied during rapid severe hemorrhage or alternatively during a period of occlusion of one or both caval veins close to the right atrium. The latter procedure was considered to mimic a rapid blood withdrawal because of the consequent rapid and considerable pooling of blood in the peripheral circulation. — The more precise location of the receptors in the heart was then established by stroking the exposed heart with a plastic rod and by application of nicotine in the pericardial cavity. The nicotine was administered in doses of 50 μg (expressed as base) dissolved in 1 ml saline. Care was taken to avoid overflow of the drug into the pleural cavity. The pericardium was afterwards rinsed with bodywarm saline. — The receptor responses to asphyxia lasting between 30—90 s were made by switching off the respirator.

To avoid disturbing muscle movements most animals were given flaxedil 2—3 mg/kg i.v.

Results

With the present experimental set up the dominating activity in cervical vagal filaments arose from atrial receptor afferents. In an initial series of experiments 60 single afferent fibres from atrial receptors of A and B type as well as of intermediate types (according to Paintal's (1953) classification) were analysed with regard to discharge characteristics during a rapid blood withdrawal. It was found that receptors of the B type regularly decreased their activity during bleeding while receptors of the A type in many cases increased their activity at least in the later stages of the bleeding period. Many receptors changed their discharge characteristics from B-type to an A type of firing during a progressive blood loss. — As evident from the spike counter there was, however, an overall decrease of atrial receptor activity with bleeding and there was in no case any sudden change in receptor associated with the appearance of the reflex bradycardia. It therefore seems reasonable to conclude that atrial receptors are not responsible for the initiation of the reflex cardial slowing during severe hemorrhage.

Besides the atrial afferents filaments displaying a very low spontaneous activity and with usually small spikes were found in the cervical vagi. These filaments sometimes showed a marked increase of activity in connection with hemorrhage as illustrated in Fig. 1. Arterial blood pressure, heart rate and spike frequency in the filament are followed during stepwise reductions of blood volume. The neurogram recorded at times 1 and 2 respectively shows that the filament contains afferents from 3 or 4 receptors exhibiting a low spontaneous activity without cardiac rhythm. With progressive bleeding there is a slight diminution of receptor activity and a moderate increase of heart rate but at a certain point there is a prompt and marked increase of impulse activity and simultaneously a slowing of the heart. With transfusion of the shed blood there is an instantaneous cessation of receptor activity and the heart rate is simultaneously increased. — The heart rate responses to an increased receptor activity were abolished after bilateral vagotomy (right vagus cut

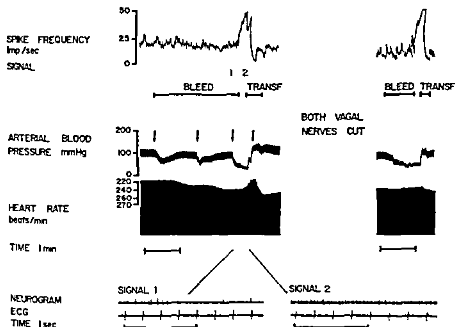


Fig 1 *Left panel* Effects of repeated hemorrhages (3 left arrows) on blood pressure heart rate and impulse activity in a vagal filament containing at least three active cardiac receptor afferents Neurograms recorded at times 1 and 2 are also shown. Note the sudden increase of nerve activity and concomitant slowing of the heart when blood volume is critically reduced — With retransfusion (right arrow) receptor activity instantaneously returns to normal and the heart rate simultaneously increases.

Right panel Cutting of the vagal nerves eliminates the bradycardial response to receptor activation during hemorrhage

centrally to the recording electrode) proving its reflex nature The receptors described in Fig 1 did not respond to asphyxiation of the animal for 30—40 sec but showed an increased activity when the ascending aorta was occluded

A total of 48 filaments containing afferents of the type described in Fig 1 were subsequently subjected to analyses Of the filaments studied 12 contained one single functioning afferent 23 filaments included 2—3 active afferents which could often be distinguished because of differences in spike heights 13 filaments included more than 3 afferents The receptors showed a very low spontaneous activity usually below 2 imp/s and often without cardiac rhythm About one third of the receptors were totally silent during resting conditions — With a partial occlusion of the aorta there was an increased activity in all studied filaments and in all clearly distinguishable receptors In the multifibre filaments it was however often impossible to follow the behaviour of each single receptor particularly when differences in spike configuration were small and fibre activity high It seems reasonable to assume however that aortic occlusion caused an increased activity in most if not all receptors subjected to study

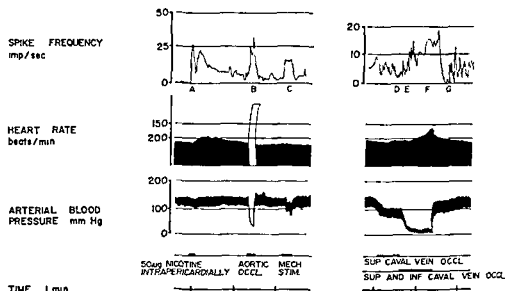


Fig 2 A Effects of nicotine aortic occlusion mechanical stimulation of the heart and occlusion of one and both caval veins on blood pressure heart rate and impulse activity in one single cardiac receptor afferent

When the effectively circulating blood volume was reduced either by rapid hemorrhage of approximately 25 % of the estimated blood volume or by occluding one or both caval veins with consequent pooling of blood in the peripheral circulation there was at a certain stage of blood loss a sudden increase of activity in 29 of the studied filaments (cf Fig 1 and 2), corresponding to approximately 20 % of the individual receptors The discharge increased to 5—22 imp/s (average 13 imp/s) with reductions of central blood volume In the multifibre filaments it was often found that there was an overall decrease of activity with progressive bleeding or pooling and that when blood volume was critically reduced the firing suddenly increased in one or sometimes two but rarely in all individual afferents in the preparation Some of the receptors showed when excited, at least initially a clearcut cardiac rhythm but the discharge usually became continuous with further blood withdrawal — With transfusion of the shed blood most receptors showed an abrupt cessation of activity and many became completely silenced for a short period before assuming the normal spontaneous resting activity again (see Fig 1)

A typical receptor response to caval occlusion is demonstrated in Fig 2 A (right panel) where alterations in blood pressure heart rate and impulse activity in a single vagal afferent were followed Neurograms taken at times indicated by letters are illustrated in Fig 2 B To establish the exact position of the receptor the responses to nicotine administration in the pericardial cavity to aortic occlusion and to a mechanical stimulation of the epicardial surface of the heart were also tested (Fig 2 A left panel) It is seen that the receptor fires with a low spontaneous activity

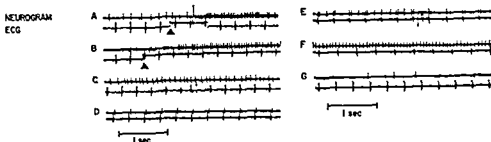


Fig 2 B Neurograms recorded at times indicated by letters in Fig 2 A

with no cardiac rhythm (*e g* left part of neurogram A) With moderate pooling (one caval vein occluded) there is, besides a fall in blood pressure a slight reduction in receptor activity which however now displays a cardiac rhythm with one spike shortly after the QRS complex in the ECG (neurogram D Fig 2 B) With a severe reduction of venous return (both caval veins occluded) there is a sudden increase of receptor activity initially with a maintained cardiac rhythm (neurogram E) but at maximal activation with a continuous discharge (neurogram F) Associated with the increased receptor discharge there is also a bradycardia probably partly of reflex origin — When the caval occlusion is released the receptor shows a two-phasic response with an initial marked reduction of spike number (neurogram G) followed by an overshoot in receptor activity probably due to a distension of the ventricles when blood is rapidly emptied into the heart from the over filled venous reservoir Such a two phasic response was often seen in experiments with caval occlusion

The recordings in Fig 2 A (left panel) demonstrate that the receptor increases its activity markedly when nicotine is injected into the pericardial cavity (Fig 2 B neurogram A) when the aorta is occluded (neurogram B) and when a localized area in the apical region of the left ventricle is mechanically stimulated by stroking with a plastic rod (neurogram C) Such exact determination of receptor position was not always possible however Thus only one third of the receptors showing an increased activity during hemorrhage or caval occlusion responded to mechanical probing of the epicardial surface of the heart and nicotine intrapericardially They all responded however to a firm pressure or squeezing of the left ventricle It therefore seems justified to conclude that the endings are positioned in the left ventricular wall possibly at varying depths

The receptor responses to asphyxiation of the animal was systematically studied in 14 vagal filaments None of the receptors studied displayed an increased activity during the first 40—60 s of the asphyxia period In contrast there was in two control experiments a markedly increased discharge in chemoreceptor afferents in the aortic nerve within 5 s after the onset of asphyxia

The conduction velocity in the receptor afferents was measured in one single fibre

preparation and in four multifibre filaments containing two functioning fibres The values found varied between 0 6 and 1 3 m/s (mean 1 0 m/s), indicating non medullated C fibres

As mentioned, an increased receptor discharge was usually accompanied by a more or less pronounced slowing of the heart Marked cardiac responses with a reduction of heart rate by 30 beats/min or more was however, only occassionally obtained and particularly when the dissection and identification of the receptor afferent in the vagal stem were successful early in the experiment The reflex cardiac responses were usually drastically reduced if the preparation procedures were prolonged over several hours before a suitable filament was found This reduced reflex responsiveness of the heart was probably in part due to unavoidable injuries of afferent and efferent pathways in the vagus during the extensive dissection Also it may be due to a deterioration of the medullary reflex centres in the later stages of the experiment since then stimulation of arterial baroreceptors produced only minor effects on the heart despite intact efferents in the left vagus nerve Furthermore when flaxedil was given the vagolytic action of this drug may also have contributed to weaken the vagal influence on the heart

Discussion

In the present study attempts were made to identify the receptors responsible for initiating the reflex slowing of the heart sometimes seen when cats are bled rapidly and severely (Öberg and White 1970) For this purpose reflex heart rate changes and afferent impulse traffic in thin vagal filaments were recorded simultaneously during rapid blood withdrawal or peripheral pooling of blood This approach presupposes that impulse recordings were made from only a small portion of afferents subserving the reflex cardiac response while the majority of the reflex pathways remained intact to allow for clearcut reflex effects

The experiments have shown that associated with and slightly preceding the reflex cardiac slowing during rapid bleeding or pooling there was an increased activity in cardiac receptors located mainly in the left ventricle and firing in non medullated vagal afferents With retransfusion of blood or cessation of pooling the receptor activity promptly declined and the heart rate simultaneously increased again

That the majority of the presently analysed receptors were located in the left ventricle seems evident from the findings that they regularly increased their activity with aortic occlusion and usually although not consistently when localized areas of the left ventricular surface were mechanically stimulated Since however such exact description of the localization of each individual receptor was often impossible in experiments with multifibre preparations particularly when their respective afferent fibre showed almost identical spike configurations in the neurogram the possibility remains that some of the studied receptors are situated in places outside the left ventricle as e g in the right ventricle or even in extracardiac structures Such

an admixture of activity in extracardiac receptor afferents is however, probably insignificant in the present recordings since the afferent pathways from arterial baro- and chemoreceptors from visceral receptors and from most lung receptors were cut distally to the recording site

Since interruption of the afferent pathways from the present ventricular receptors by cutting the cardiac nerves leads to a disappearance of the reflex bradycardia during hemorrhage (Öberg and White 1970) it seems reasonable to conclude that they are to a large extent responsible for triggering this response

It should however be noted that the vagal filaments recorded from in the present experiments were not randomly sampled but rather selected according to their property to show an increased total activity when aorta was occluded Such a selection procedure must necessarily lead to an over representation of left ventricular receptor afferents in the studied nerve bundles One can therefore not exclude that also other cardial receptors which because of their location *e g* in the right ventricle do not respond significantly to aortic occlusion and therefore escaped further analyse in the present study also contributed to the reflex cardiac response during hemorrhage It seems clear however that the classical atrial receptor of A and B type play no part in this reflex response Evidently some of the left ventricular receptors activated during hemorrhage were rather superficially located in the ventricular wall since they responded to a moderately strong stroking across the ventricular surface and also to nicotine administered locally in the pericardial cavity Other receptors were not excited by either one of these manoeuvres but were activated by a more firm squeezing of the ventricle and were therefore probably positioned in deeper layers of the ventricular wall

The receptors showed a very low spontaneous discharge usually below two impulses per second Approximately 30 % of the studied receptors were quite silent during normal conditions The firing was as a rule quite irregular with no evident cardiac rhythm — When the receptors became excited however they often showed a cardiac rhythm They were not activated by a short lasting asphyxia which caused arterial chemoreceptors to fire heavily The receptors can therefore be classified as mechanoreceptors rather than chemoreceptors

The natural stimulus for the receptors seems to be a distortion of the ventricle wall caused either by distension as during aortic occlusion or by contractions of the ventricles around an almost depleted chamber as with rapid blood withdrawal or peripheral pooling of blood As mentioned however only 20 % of the receptors responding to aortic occlusion were excited during hemorrhage or pooling This is probably explained by a less efficient receptor stimulation caused by the latter procedures rather than being any indication of two types of functionally different receptor groups Thus it was sometimes found that a receptor which at one time responded to a depletion of the intrathoracic blood reservoirs did not respond when this procedure was repeated somewhat later in the experiment A substantial receptor activation and a pronounced reflex bradycardia (Öberg and White 1970) was as a rule seen only when there was evidence of a strong sympathetic excitation of the

heart in terms of a marked initial tachycardia during blood withdrawal It therefore seems as if an extremely poor diastolic filling of the heart when combined with vigorous contractions of the ventricles creates a situation of tearing and squeezing of the myocardium which acts as a stimulus for the described ventricular receptors — In the absence of a strong inotropic influence there was usually no receptor activation or reflex bradycardia during blood withdrawal

The reflex circulatory adjustment elicited from the present receptors were presently not analyzed except for the effects on heart rate Receptor activation was thus usually accompanied by a reflex bradycardia, although admittedly rather moderate in most of the experiments This rather weak reflex influence on the heart rate does not disqualify the receptors as triggers of the reflex bradycardia during hemorrhage As mentioned the absence of a marked reflex vagal influence on the heart could in the present experiments usually be explained by deterioration of the reflex mechanism since other procedures known to produce a vagal slowing of the heart were then also incapable to reduce heart rate significantly The more intact the preparation was and therefore comparable to that used by Öberg and White (1970), the more pronounced were the reflex bradycardial responses

Cardiac receptors resembling the present ones have been reported by other authors Thus Jarisch and Zotterman (1948) described receptors signalling in slow conducting probably non medullated afferents and responding to mechanical stimulation of the ventricles and to occlusion of the aorta More recently non medullated afferents from heart receptors have been reported in dogs by Coleridge Coleridge and Kidd (1964) and by Sleight and Widdicombe (1965) One group of endocardial myocardial endings described by the latter authors were sensitive to nicotine mechanical of the heart and aortic occlusion, and are therefore probably identical to those presently described in the cat

The bradycardia reflexly produced when the present receptors are excited resembles in many respects the so called vaso vagal syncope reaction in man (e g Barcroft and Swan 1953) It has indeed been proposed that this reaction is initiated from receptors located in the heart (e g Jarisch 1941 Henry 1955 Sharpey Schaefer 1956) The present study thus furnishes electrophysiological evidence to support this hypothesis Pearce and Henry (1955) described an increased activity in atrial receptor afferents after bleeding and intravenous adrenaline in huge doses but no attempt was evidently made to correlate this receptor activity to alterations in heart rate It is conceivable that a vaso vagal syncope reaction is preceded by a continuous diminuition of the diastolic filling of the heart and an intensified sympathetic stimulation of the ventricles until such a situation is created that due to a squeezing effect the receptors are suddenly activated The induced reflex bradycardia will then allow for a better diastolic filling of the heart and possibly improve its pumping efficiency The harmful effects of the tearing and squeezing of the myocardium manifesting itself as subendocardial hemorrhages (Gauer and Henry 1964) will also be offset by this powerful reflex break on the pump — The present receptors and the vaso-vagal reaction may therefore be ascribed a protective func

tion through which the diastolic filling and contractile force of the heart are matched in situations of a critically reduced venous return

A cholinergic vasodilatation has been proposed to occur in human skeletal muscles during posthemorrhagic fainting (Barcroft and Edholm 1945) It is therefore of interest that Bergel and Makin (1967) claim that stimulation of cardiac receptors probably similar to the present ones by applications of nicotine in the pericardial cavity causes a reflex activation of the sympathetic cholinergic vasodilator fibres to the skeletal muscle Whether such a mechanism really contributes to the emergence of a vaso vagal syncope needs to be further elucidated

The functional characteristics of the present receptors will be further discussed in a subsequent publication

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Acta physiol scand 1972 85 174—182

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The Influence of Deep Barbiturate Anesthesia upon the Regulation of Extra- and Intracellular pH in the Rat Brain during Hypercapnia

By

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Abstract

MESSETER K U PONTÉN and B K SIESJO *The influence of deep barbiturate anesthesia upon the regulation of extra and intracellular pH in the rat brain during hypercapnia* Acta physiol scand 1972 *85* 174—182

In order to study the influence of deep barbiturate anesthesia upon the regulation of extra and intracellular pH in the brain hypercapnia was induced in rats anesthetized for maximally three hrs with either 200—250 mg/kg of phenobarbital or with 0 6 halothane There were no differences in the regulation of the CSF pH between the groups studied However both at 15 min and at 3 h the phenobarbital anesthesia was associated with a less efficient regulation of the intracellular pH At 15 min the regulation observed came close to that expected on the basis of pure physicochemical buffering It is concluded that deep phenobarbital anesthesia probably due to its effect on metabolism can interfere with the mechanisms which regulate intracellular pH during hypercapnia

It has recently been shown that when rats are made hypercapnic by means of administration of about 11 % CO_2 there is during the first three hours a gradual increase in the intracellular bicarbonate concentration of such a magnitude that the intracellular pH is brought back to within a few hundredths of a pH unit from the normocapnic control value (Messeter and Siesjo 1970 1971 a) An analysis of the factors responsible for the regulation of pH$_i$ indicates that the increase in the HCO_3^- concentration during the first 45 min is mainly due to physicochemical buffering and to consumption of metabolic acids like lactic pyruvic α ketoglutaric and glutamic acid while the additional increase in the HCO_3^- concentration observed at 3 h may be largely due to transmembrane fluxes of H^+ or HCO_3^- ions (Siesjo and Messeter 1971 Siesjo *et al* 1972) There is also some evidence indicating that any such flux must occur against an electrochemical gradient, *i e* that it occurs at the expens of metabolic energy (Siesjo and Messeter 1971)

The maximal pH regulation observed in the above experiments was obtained already at 3 h in that prolongation of the hypercapnia to 72 h did not lead to further

intracellular acid base changes (Messeter and Siesjo 1971 a) In all these latter groups including the 3 h group the pH regulation pertained to the unanesthetized state since anesthesia with 0 6—0 7 % halothane was not induced until 30—45 min before the end of the exposure periods In the 15 and 45 min groups however the animals were kept anesthetized during the whole exposure periods

The present experiments were undertaken to study if reduction in cerebral metabolic rate induced by anesthesia and particularly by deep barbiturate anesthesia would influence the rate of accumulation of intracellular bicarbonate during hypercapnia Therefore rats were exposed to about 11 % CO_2 for up to 3 h in the anesthetized state and the anesthesia was either light (0 6 % halothane) or very deep (200—250 mg/kg of phenobarbital) Acid base parameters were evaluated in arterial blood cisternal cerebrospinal fluid and brain tissue and the results were compared to those previously obtained from unanesthetized animals

Methods

Operative and sampling techniques All experiments were performed on male wistar rats weighing 300—400 g which were tracheotomized immobilized with tubocurarine chloride and ventilated on a Starling type respirator The body temperature was measured in the rectum and maintained close to 37 C One femoral artery was cannulated for blood pressure recording and for repeated anaerobic sampling of arterial blood Immediately after sampling the blood was analysed for pH P_{CO_2} and P_O and for the hemoglobin content

In a number of control experiments a hole was drilled in the skull bone over the superior sagittal sinus and cerebral venous blood was sampled in heparinized glass capillaries for subsequent measurements of the CO tension (see Ponten and Siesjo 1966 Brzezinski *et al* 19[illegible]7)

At the end of the experiment cisternal cerebrospinal fluid (CSF) was sampled by puncturing the atlantooccipital membrane with the sharp tip of a glass capillary The brain was then frozen *in situ* by pouring liquid nitrogen into a plastic funnel fitted into a skin incision over the cranial vault The supratentorial parts of the brain were chiselled out in the frozen state and stored at −85 C until analyzed (see Pontén 1966 Messeter and Siesjo 1971a)

The animals were either anesthetized with 0 6—0 7 [illegible] halothane or with phenobarbital in a dose of 200—250 mg/kg i p A few animals were anesthetized with 70 [illegible] N O instead of 0 6 [illegible] halothane but since the results were identical the animals were grouped together In the phenobarbital group the majority of the animals were given 250 mg/kg i p This dose is associated with a fall in blood pressure to about 80 mm Hg (see Nilsson and Siesjo 1970) For that reason some animals were given a dose of 200 mg/kg However since the results obtained with the two doses were identical except for a small difference in the arteriovenous P_{CO_2} differences (see below) all phenobarbital animals were grouped together In the halothane groups anesthesia was initiated with divinyl ether in a closed jar (Nilsson and Siesjo 1970) but in the phenobarbital groups the tracheotomy was performed when surgical anesthesia was attained on the barbiturate In all groups the inhaled oxygen concentration was kept at about 30 [illegible] In the majority of experiments the arterial oxygen tension was therefore well above 100 mm Hg and in no case was the P_aO lower than 80 mm Hg All animals were allowed a steady state period of 15—30 min before hypercapnia was induced by means of the administration of about 11 [illegible] CO When the animals were connected to the CO containing gas mixture mechanical hyperventilation was started. The hyperventilation [illegible] of a magnitude to reduce the arterial CO tension to 15—20 mm Hg in air breathing animals [illegible] used to ensure a rapid equilibration with the inhaled CO Previous results and control experiments [illegible] have shown that with this degree of overventilation a new steady tissue CO tension is reached [illegible] about 5 min (Siesjo 196[illegible]

Analytical techniques Immediately after sampling arterial blood was analysed for pH [illegible] P_{O_2} using microelectrodes operated at 37 0 C Radiometer Copenhagen and [illegible] Kiel) and for the hemoglobin content, using a [illegible] tat on Hb-meter The pH P_{CO_2} and [illegible] were corrected for deviations in body temperature from 37 0 C. The pH values were [illegible] equimolar phosphate buffers of the National Bureau of Standards (pH 6 813 and 7 [illegible] at 37 0 C) The CO and O electrodes were repeatedly checked by using blood tonometered at 37 0 C with known carbon dioxide and oxygen tensions

The CSF samples were analysed for the CO_2 content immediately after sampling using a modified Conway micro-diffusion technique (Siesjo 1962a) The method was checked by titrating a 0 025 M Na CO solution which was delivered from the same type of glass capillaries as was used for CSF *Due to the small CSF volumes obtained (60—100 µl) only single samples could be analysed* However when Na_2CO solutions of equal volume were analysed the means of a series of samples were always within 1 % of the gravimetric standard and individual samples very seldom deviated more than 2 from the mean value

The tissue samples were crushed in liquid nitrogen in a CO free atmosphere and aliquots of the crushed tissue were analysed for the CO content using the method described by Pontén and Siesjo (1964) The principal features of the method were as described in that paper but the diffusion *units are now modified (Siesjo and Thompson 1965) The samples from each brain were titrated* against a blank sample The method was intermittently checked by titrations of a 0 025 M Na CO solution which was frozen in liquid nitrogen and treated as the tissue samples The mean difference between duplicate analyses was usually about 0 5 % and the estimated accuracy of the method is 1 or better

Calculations The calculations of the intracellular bicarbonate concentration and of intracellular pH was made according to the equations

$$[HCO^-_3]_t = T_{CO} - P_{tCO} \; 0\,0292$$

$$[HCO^-_3]_i = \frac{[HCO^-_3]_t - 0\,15[HCO^-_3]_{CSF} - 0\,03[HCO^-_3]_{bl}}{0\,61}$$

$$pH_i = 6\,120 + \log \frac{[HCO^-_3]_i}{P_{tCO} \; 0\,0314}$$

In these equations $[HCO^-_3]_t$ is the tissue HCO^-_3 concentration (mEq/kg wet tissue) P_{tCO_2} the mean tissue CO tension (mm Hg) $[HCO^-_3]_i$ the intracellular HCO concentration (mEq/kg i c water) 0 15 $[HCO^-_3]_{CSF}$ and 0 03 $[HCO^-_3]_{bl}$ the amount of bicarbonate contained in the extra cellular fluid and blood volumes of the tissue which were thus assumed to occupy 15 and 3 respectively of the tissue weight 0 61 the intracellular volume (g i c H_2O/g tissue) 0 0292 and 0 0314 the CO_2 solubility coefficients for wet tissue and intracellular wtaer respectively (mmol kg⁻ mm Hg) and 6 120 the pK of carbonic acid (see Siesjo 1962 a and b Pontén 1966 Kjällquist *et al* 1969 Siesjo and Messeter 1971 Messeter and Siesjo 1971 a)

The figure for the size of the extracellular space (15) is in accordance with recent reevaluations of the inulin space of the brain (Woodward *et al* 1967 Rall and Fenstermacher 1971) It was assumed that the extracellular fluid and blood volumes of the tissue remain the same in hypercapnia This appears to be a valid assumption for the ECF volume (Cameron *et al* 1970) but since the blood volume may increase somewhat in hypercapnia the intracellular HCO^- concentrations could be slightly overestimated

Results

Acid base changes in arterial blood In the experiments to be reported the mean arterial blood pressure was around 145 mm Hg in the groups of animals anesthetized with halothane (or N_2O) In the phenobarbital groups the mean arterial blood pressure was around 100 mm Hg and in no single experiment was the blood pressure lower than 80 mm Hg The mean hemoglobin concentration varied between 13 6 and 17 5 g/100 ml There were no signs of hemodilution or hemoconcentration during the 3 h experiments irrespective of the anesthetic used

In the animals which were anesthetized for 3 h at least 4 sets of blood samples were taken Table I gives for the normocapnic animals the acid base parameters measured during the first 20 min after the end of the operative procedures about 1 h later and at the end of the 3 h period In the hypercapnic animals the values recorded are those measured 1—1 5 h after the end of the operation and at 3 h In the animals which were anesthetized with halothane (or nitrous oxide) the 3 h anesthetic period gave no measurable acid base changes ($0\,4 < p < 0\,5$) However the

TABLE I The measured arterial pH and P_{CO} and the calculated $[HCO^-]$ in groups of rats anesthetized with 0 6 % halothan during 3 h of normocapnia or hypercapnia compared to similar groups of animals anesthetized with phenobarbital (250 mg/kg) During 3 h of anesthesia the phenobarbital injected groups developed a moderate non respiratory acidosis which was not observed in the halothane material Number of experiments within parentheses Means ± S E

Exposure period	Halothane			Phenobarbital		
	PaCO mm Hg	pH	$[HCO^-]$ mEq/kg	PaCO mm Hg	pH	$[HCO^-_3]$ mEq/kg
Normocapnia						
15–20 min	37 3 ±0 8 (8)	7 41 ±0 01	2? 7 ±0 5	37 3 ±1 1 (7)	7 44 ±0 01	2? 7 ±0 6
60 min	37 7 ±0 9	7 41 ±0 01	23 0 ±0 4	37.2 ±1 2	7 39 ±0 0?	21 9 ±0 6
180 min	37 5 ±1 1	7 40 ±0 01	22 6 ±0 5	39 0 ±0 9	7 30 ±0 01	19 1 ±0 7
11 CO						
60 min	86 8 ±1 3 (9)	7 14 ±0 01	27 9 ±0 6	89 7 ±3 8 (6)	7 10 ±0 00	?6 7 ±1 2
180 min	88 5 ±1 3	7 14 ±0 01	28 5 ±0 8	90 5 ±2 8	7 10 ±0 02	26 5 ±0 7

phenobarbital anesthesia was associated with a significant nonrespiratory acidosis at 3 h with a decrease in pH of 0 1 units ($p < 0\ 001$) and in the plasma HCO^-_3 concentration of 4 mEq/l ($p < 0\ 01$)

In hypercapnia there was a similar although somewhat smaller difference between the anesthetic groups Thus whereas there was an *increase in the plasma* HCO^-_3 concentration in the phenobarbital group at 1–1 5 h roughly comparable to that measured in the group anesthetized with halothane or nitrous oxide the phenobarbital animals accumulated 2 mEq/l HCO^-_3 less at 3 h

Arteriovenous differences in CO_2 *tensions* It has previously been shown that the mean tissue (and CSF) CO_2 tensions can be calculated by adding about 0 6 mm Hg to the arithmetic mean of the arterial and cerebral venous CO_2 tensions (Ponten and Siesjo 1966) In order to allow calculations of the tissue CO_2 tension from the arterial CO_2 tensions arteriovenous P_{CO_2} differences were repeatedly measured in animals anesthetized with either 200 mg/kg or with 250 mg/kg of phenobarbital 24 measurements in 6 normocapnic animals ($PaCO_2$ 34 9–41 0 mm Hg) anesthetized with 250 mg/kg gave a mean P_{CO_2} difference of 15 4 mm Hg (S E ±0 3 mm Hg) In the 200 mg/kg group ($PaCO_2$ 34 2–42 5 mm Hg) the corresponding mean value for 14 measurements in 4 animals was 13 3 mm Hg (S E ±0 3 mm Hg) The differences between the groups were statistically significant ($p < 0\ 001$) In the 250 mg/kg animals the mean tissue (and CSF) CO_2 tension was therefore calculated by adding 8 2 mm Hg to the arterial CO_2 tension and in the 200 mg/kg animals by adding 7 2 mm Hg In hypercapnia ($PaCO_2$ 78 1–91 6 mm Hg) 13 measurements in 4

TABLE II The measured CO_2 content and calculated acid base parameters in the CSF during normocapnia (30–45 min or 3 h) and hypercapnia (15 min or 3 h) in groups of rats anesthetized with 0 6 % halothane (or N_2O) as compared to animals anesthetized with phenobarbital (250 mg/kg) Although prolonged anesthesia with phenobarbital was accompanied by a moderate acidosis under normocapnic conditions (3 h) the regulation of the CSF pH in acute and sustained hypercapnia was identical in the 2 groups of animals Number of experiments within parenthesis Means ± S E

	Halothane				Phenobarbital			
Exposure period	PtCO mm Hg	T_{CO_2} mEq/kg	[HCO⁻] mEq/kg	pH	PtCO mm Hg	T_{CO_2} mEq/kg	[HCO⁻₃] mEq/kg	pH
Normocapnia								
30–45 min	44 4 ±0 8 (9)	29 7 ±0 4	28 3 ±0 4	7 43 ±0 01	46 8 ±0 7 (7)	28 8 ±0 5	27 3 ±0 5	7 39 ±0 01
180 min	43 5 ±1 1 (7)	29 9 ±0 6	28 5 ±0 6	7 44 ±0 01	47 2 ±0 9 (7)	27 4 ±1 0	25 9 ±1 0	7 37 ±0 01
II ‘ CO								
15 min	87 5 ±1 9 (4)	35 0 ±0 3	3’3 ±0 3	7 20 ±0 01	87 7 ±1 4 (6)	33 6 ±0 4	30 9 ±0 4	7 17 ±0 01
180 min	93 7 ±1 6 (6)	42 8 ±1 0	39 9 ±0 9	7 26 ±0 01	96 2 ±2 8 (5)	41 2 ±0 7	38 1 ±0 7	7 23 ±0 07

animals anesthetized with 250 mg/kg phenobarbital gave a mean a v P_{CO} difference of 10 3 mm Hg (S E ±0 6 mm Hg) In these animals the tissue CO_2 tension was therefore obtained by adding 5 7 mm Hg to the $PaCO_2$ It should be observed that the derived CSF arterial P_{CO} differences measured in deep phenobarbital anesthesia were about 2 mm Hg higher than those previously obtained in halothane anesthesia (Messeter and Siesjo 1971 b) The differences previously measured (6 5 and 4 5 mm Hg respectively) were used to calculate the tissue and CSF CO_2 tensions in the present normocapnic and hypercapnic groups anesthetized with halothane

Acid base changes in cisternal CSF Table II gives the CSF acid base parameters in the normocapnic animals measured 30–45 min and 3 h respectively after the end of the operative procedures In hypercapnia groups of animals were studied 15 min as well as 3 h after the beginning of the CO_2 administration

3 h of anesthesia with halothane or nitrous oxide did not lead to any changes in the CSF acid base parameters With phenobarbital anesthesia the CSF HCO_3^- concentration and the pH appeared slightly lower and this relative CSF acidosis was exaggerated after 3 h of anesthesia (*c f* changes in arterial blood) However the phenobarbital anesthetized animals showed the same degree of pH regulation during 3 h of hypercapnia as did the lightly anesthetized halothane animals In order to get an expression for the degree of pH regulation which is independent of variations in the CO_2 tension the percentage pH regulation calculated as

$$\% \text{ pH reg} = \frac{\log [HCO_3^-]_2 - \log [HCO_3^-]_1}{\log P_{CO_2} - \log P_{CO_2}} \cdot 100$$

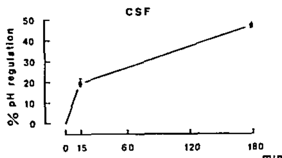


Fig 1 The percentage pH regulation (see text) in the CSF in groups of rats anesthetized with 0 6 halothane (unfilled circles) or with 250 mg/kg of phenobarbital (filled circles) and exposed to about 11 CO for 15 min and 3 h respectively The observed pH regulation was identical with the two types of anesthesia (Means ± S E.)

(Siesjo 1971) has been shown in Fig 1 In the equation the superscripts ( ) and ( ) denote the hypercapnic and normocapnic (45 min) conditions respectively There was an identical degree of pH regulation in the groups ($0\ 8 < p < 0\ 9$) and thus no indication that deep phenobarbital anesthesia interfered with the accumulation of HCO^-_3 in the CSF during hypercapnia

Intracellular acid base changes Table III gives the intracellular values which corresponded to the CSF values shown in Table II Three h of anesthesia with halothane or nitrous oxide during normocapnia did not lead to any changes in the intracellular acid base parameters but prolonged phenobarbital anesthesia appeared to cause a slight shift in the acid direction ($0\ 01 < p < 0\ 02$) It should be observed that deep phenobarbital anesthesia significantly shifts the intracellular pH in the alkaline direction and the normocapnic value at 30—45 min is in very good agreement with

TABLE III The influence of halothane (0 6) and phenobarbital anesthes a (250 mg/kg) upon the measured brain tissue CO content and calculated intracellular ac d base parameters in groups of rats during normocapnia or hypercapnia Phenobarbital anesthes a was as ociated with a moderate decrease in the pH_i during 3 h of normocapnia and with a less efficient regulat on in acute and ustain d hypercapnia as compared to the halothane material Number of experiments within parenthesis Means ± S E

Exposure period	Halothane PtCO mm Hg	Halothane T_{CO} mEq/kg tissue	Halothane $[HCO\ ]_i$ mEq/kg i c water	Halothane pH_i	Phenobarbital PtCO mm Hg	Phenobarbital T_{CO_2} mEq/kg tissue	Phenobarbital $[HCO^-\ ]_i$ mEq/kg i c water	Phenobarbital pH_i
Normocapnia								
30–45 min	43 9 ±0 6 (14)	13 4 ±0 2	12 0 ±0 3	7 06 ±0 01	46 8 ±0 7 (7)	15 5 ±0 3	15 6 ±0 4	7 15 ±0 01
180 min	44 6 ±1 3 (6)	13 7 ±0	12 3 ±0 2	7 07 ±0 01	47 2 ±0 9 (7)	14 5 ±0 4	14 4 ±0 4	7 11 ±0 01
11 CO								
15 min	88 9 ±1 1 (10)	18 8 ±0 2	18 0 ±0 3	6 93 ±0 01	87 7 ±1 4 (6)	20 1 ±0 3	20 1 ±0 3	6 93 ±0 01
180 min	93 1 ±1 6 (9)	22 8 ±0 3	22 0 ±0 4	7 00 ±0 01	96 2 ±2 8 (5)	23 5 ±0.2	23 4 ±0 3	7 01 ±0 02

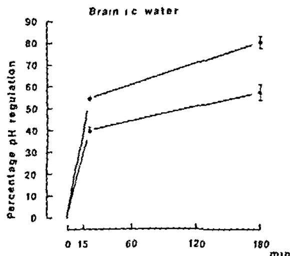


Fig 2 The calculated percentage pH regulation in brain i c water in groups of rats anesthetized with 0 6 % halothane and exposed to 11 % CO_2 for 15 min and 3 h respectively (unfilled circles) as compared to similar groups of animals anesthetized with phenobarbital in a dose of 250 mg/kg (filled circles) There was a less efficient regulation in the phenobarbital material (Means ± S E)

that previously measured (Nilsson and Siesjo 1970) In halothane anesthesia there was an efficient regulation of pH i during the 3 h of hypercapnia comparable to that observed in the unanesthetized animal (see Messeter and Siesjo 1971 a) With phenobarbital anesthesia however there was a much less efficient regulation of pH i both at 15 min and at 3 h This is especially apparent if the percentage pH regulation is compared The data of Fig 2 which were obtained by calculating the percentage pH regulation for each individual experiment (comparing the intracellular HCO^-_3 concentrations and the CO_2 tensions to the corresponding mean values for the control group at 30–45 min) show that whereas the halothane groups gave values of 55 ± 3 and 81 ± 3 % at 15 min and 3 h respectively the phenobarbital groups gave values of 40 ± 2 and 58 ± 3 % respectively The difference in pH regulation between the halothane and phenobarbital groups respectively, was highly significant at both 15 min and 3 h ($p < 0.001$)

Discussion

The present experiments were devised to study two things Firstly, since deep barbiturate anesthesia by itself is known to decrease the steady state tissue contents of anions like lactic and pyruvic acid (Nilsson and Siesjo 1970) and since such a decrease during hypercapnia has been assumed to act as a pH regulating factor (Siesjo and Messeter 1971) we induced acute hypercapnia in deep barbiturate anesthesia with the goal of trying to approximate the true physicochemical buffer capacity of the tissue Secondly if the gradual accumulation of HCO^-_3 in the brain during prolonged hypercapnia in the unanesthetized animal depends upon metabolic energy (Siesjö and Messeter 1971) an impaired pH regulation should be expected to occur with deep barbiturate anesthesia which is known to decrease the cerebral metabolic rate for oxygen (Sokoloff 1960)

The present results demonstrated that prolonged phenobarbital anesthesia by itself seemed to lead to a lowering of the pH_i measured about 45 min after the induction of the anesthesia. This decrease could be due to the fact that the deep anesthesia interferes with a mechanism extruding H^+ ions from the cells. The results also demonstrated a markedly low degree of pH regulation in the phenobarbital animals during both acute and prolonged hypercapnia. The percentage pH regulation obtained at 15 min (about 40 %) is rather close to that expected on the basis of pure physicochemical buffering. Thus the buffer capacity derived from homogenate studies (Siesjo and Messeter 1971) corresponds to a percentage pH regulation of about 40 %. This indicates that the metabolic changes induced by deep phenobarbital anesthesia interferes with the metabolic regulation of intracellular pH in acute hypercapnia.

The relatively low degree of intracellular pH regulation which was obtained during 3 h of phenobarbital anesthesia occurred in spite of a normal CSF pH regulation as compared to superficial anesthesia with volatile anesthetics. It therefore strongly indicates that the deep barbiturate anesthesia by its effect on metabolism in some way retards the accumulation of HCO^-_3 in the intracellular fluids of the brain during hypercapnia. Although the results indicate a higher degree of intracellular pH regulation during phenobarbital anesthesia than was previously reported from this laboratory (Pontén 1966) they confirm our conclusions that deep barbiturate anesthesia is associated with a less efficient pH regulation than that observed with no or with only a superficial anesthesia (cf. Kjallquist *et al.* 1969, Granholm and Ponten 1969). In a recent publication using the DMO method for pH_i determinations (Roos 1971) the effect of anesthesia on the brain pH_i was evaluated on rats during 70 min of respiratory acidosis and alkalosis. The pH regulation in rats anesthetized with nitrous oxide was in close agreement with the present data obtained in animals anesthetized with halothane, whereas the administration of pentobarbital (40 mg/kg) did not affect regulation. Although these findings superficially do not seem to corroborate our conclusions it should be recalled that light barbiturate anesthesia may not affect the mechanisms responsible for the intracellular pH regulation (Nilsson and Siesjo 1970).

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Acta physiol scand 1972 85 183—192
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Analysis of Facial Reflex Facilitation and Inhibition by Microelectrode Recording from the Brain Stem

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Abstract

LINDQUIST CHR *Analysis of facial reflex facilitation and inhibition by microelectrode recording from the brain stem* Acta physiol scand 1972 *85* 183—192

The response in the facial nucleus to stimulation of peripheral facial and trigeminal nerve branches is described After antidromic invasion the excitability of facial motoneurons was lowered for up to 30 ms before as well as after intravenous injections of strychnine The depression following antidromic stimuli is thus not caused by postsynaptic inhibition of the Renshaw type but rather by positive afterpotentials in the motoneurons Synaptic activation by tap stimulation of the face led to increased motoneuron excitability setting in after a latency of 3 ms and lasting for 30 ms This was followed by a lowered excitability lasting for an additional period of 65 ms The period of depression was presumed to be due to postsynaptic inhibition of motoneurons The changes of the excitability of facial motoneurons following synaptic activation explain in part previously observed effects of conditioning afferent stimuli on facial reflexes —Responses to electrical stimulation of facial and hypoglossal nerve afferents were recorded from reticular neurons These neurons responded to single stimuli with a repetitive high frequency discharge related to the stimulus strength and could respond to an afferent inflow from large areas of the body and limbs It is discussed whether these reticular neurons may be interneurons in a system exerting inhibitory control of facial muscle reflexes

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Previous experiments showed that facilitation and/or inhibition of reflexes in the cat s facial muscles may be produced by conditioning mechanical stimulation of the facial skin and by conditioning electrical stimulation of afferents from various sources such as the tongue the forelimbs and even the hind limbs The facilitation was of shorter latency and duration than the inhibitory processes The depression of facial muscle reflexes by a conditioning stimulus volley could be sustained for several hundred ms The inhibitory effects of conditioning facial taps on facial reflexes were considerably diminished following intravenous injections of strychnine but the drug had no obvious effect on reflex inhibition induced by afferent volleys in the radial nerve It was concluded that two different inhibitory mechanisms are operative in the control of facial muscle reflex activity (Lindquist 1972) The time course of the reflex inhibition was similar to that for depolarization of trigeminal primary afferents by mechanical stimulation of the skin and by electrical stimulation of the radial

nerve (Darian Smith 1965 Baldissera *et al* 1967 Vyklicky *et al* 1967) This similarity suggested that one of the inhibitory mechanisms may be presynaptic To test this hypothesis picrotoxin was injected as a blocker of presynaptic inhibition These attempts gave negative results and the evidence for presynaptic inhibition in the control of facial muscle reflex activity therefore remains indirect (Lindquist 1972)

With the present series of investigations a further step has been taken in the analysis of facial muscle reflexes Results from extracellular microelectrode recordings from the facial nucleus will be presented Typical responses to antidromic and synaptic activation will be described and the excitability of facial motoneurons following such activation has been studied in order to identify the mechanisms behind the effects of conditioning stimuli on facial muscle reflexes Finally the properties of certain medullary neurons have been examined and it is suggested that these might be interneurons in a reflex arc exerting inhibitory control of the reflex activity in facial muscles

Part of the results was presented in a preliminary report (Lindquist 1970)

Methods

As experimental animals 25 cats weighing 2 5 to 3 5 kg were used Anesthesia was induced by ether and maintained by i v injections of chloralose in doses of 60—80 mg/kg b wt The cats were kept warm with an infrared heating lamp Except when muscle contractions were studied the animals were curarized with Flaxedil® or Celocurin®

Mechanical taps of 3—5 ms duration were delivered to the skin as previously described (Lindquist and Mårtensson 1970) A Grass S4 stimulator and bipolar chlorided silver wire electrodes were used to deliver stimuli of 0 1 ms duration to peripheral nerves Similar electrodes were used for recording from nerves

Extracellular recordings from the brain stem were obtained with commercially available tungsten microelectrodes lacquer isolated except for ? μ from the tip The electrode tip diameter was around 1 μ and the electric impedance about 10 MΩ at 1000 Hz (Transidyne General Corp Microtrode® model no 404 10)

The cat s head was placed in a stereotaxic instrument and the electrodes inserted from the dorsal side through a hole in the skull and the intact cerebellum at an angle of 45 to the frontal plane so as to avoid the bony tentorium

The bioelectric signals were fed into a cathode follower and a capacitance coupled Grass P6 amplifier connected to a Tektronix 502 dual beam oscilloscope and a loudspeaker

Results

Antidromic responses in the facial nucleus In Fig 1 the zygomatic branch of the facial nerve is stimulated at constant strength and the records *A*—*E* show antidromic responses recorded at five different depths as the microelectrode approaches the facial nucleus The distance from the recording site in 1 to that in *E* is 0 7 mm A hardly perceptible positive negative potential is recorded in *A* This response in particular its negative component increases in amplitude as the microelectrode is pushed ventrally (*B* and *C*) and reaches a maximum in *E* The response latency is 1 ms and the duration of the maximal response in *F* is 2 5 ms In other cases potentials lasting up to 4 ms were recorded As the stimulus strength was increased and additional motor fibers were recruited the responses in Fig 1 increased in amplitude indicating that the microelectrode was recording the compound response from





Fig 1 Fig 2

Fig 1 A–E Antidromic responses recorded in brain stem on stimulation of zygomatic branch of facial nerve Full description in text Time bar 2 ms

Fig 2 A and B antidromic responses in facial nucleus to two consecutive stimuli of equal strength applied to zygomatic branch of facial nerve in non strychninized cat C experiment in A repeated after strychnine injection ([illegible] mg/kg b wt.) Stimulus interval in A and C [illegible]0 ms in B 100 ms Time bars 10 ms

several motoneurons Typical responses like that in E were usually characterized by an inflexion on the leading front Sometimes responses of short duration and with a steep leading front without any inflexion were seen This latter type of potential may originate in axons whereas the former may be soma potentials (cf Frank and Fuortes 1955)

Antidromic field potentials similar to those illustrated in Fig 1 were elicited in the facial nucleus on stimulation of the ventral facial branch as well The maximal responses were then obtained slightly more ventrally

When the electrode was in position to record the response in E a 0 1 ms long electric pulse was applied through it This resulted in an orthodromic impulse volley that could be recorded in the zygomatic branch at stimulus strengths below 50 µamp The threshold for elicitation of contractions in the upper lip of non curarized cats was also minimal in this area The lowest threshold for evoking contractions in the muscles of the pinna and in the digastric muscle was found about 1 mm more medially These findings suggest a somatotopical organization of the motoneurons in the facial nucleus which is in accordance with results from histological studies (Courville 1966)

Some experiments were terminated by allowing a coagulating direct current to pass through the recording microelectrode when this was in the optimal position for recording antidromic field potentials The microelectrode site in the facial nucleus could then be verified by microscopical examination of sections from the brain stem



Fig 3 Field potential evoked in facial nucleus in response to stimulation of ipsilateral infra orbital nerve Full description in text Time bar 5 ms

Measurements of the peripheral conduction distance and the latency of anti dromic potentials showed that the stimulus strengths used elicited impulses travelling in motor fibers at a calculated conduction velocity of 35 to 65 m/s

Excitability of facial motoneurons following antidromic invasion Excitability changes in motoneurons are most readily studied in intracellular recordings but this technique proved to be difficult to apply in long lasting studies of neurons located in the brain stem due to vascular pulsations The amplitude of an antidromic response can also be used as index of the excitability in a cranial motoneuron pool (Morimoto *et al* 1968) and since this method is more convenient and has previously been shown to yield valuable information it has been used in the present investigations of moto-neuron excitability

The excitability of the motoneuron pool following antidromic activation was studied in order to see whether there is a self regulation of the efferent impulse flow from the facial nucleus To this end an antidromic test stimulus was applied at various intervals after antidromic stimulation of a facial nerve branch The recordings in Fig 2 *A* and *B* are from such an experiment in which the zygomatic branch is stimulated In *A* the antidromic test stimulus is trailing an antidromic conditioning stimulus by 20 ms The test response is of lower amplitude than the preceding conditioning response Such a decreased excitability in the facial nucleus was regularly observed up to 30 ms after an antidromic invasion A lowered excitability of more than 30 ms duration was more seldom recorded In *B* however a smaller antidromic response results when the test stimulus is trailing the conditioning shock by 100 ms

Earlier studies of spinal motoneurons have shown that the depression of moto neuron excitability following antidromic stimulation is caused by two factors positive afterpotentials and postsynaptic inhibition of the Renshaw type (see *e g* Brooks and Wilson 1959) One or both of these mechanisms should also explain the depression of facial motoneuron excitability after antidromic excitation At the spinal level strychnine blocks postsynaptic inhibition and this drug was therefore injected in order to evaluate the relative importance of the two possible mechanisms The experiment in Fig 2 *A* was repeated after strychninization and as appears from Fig 2 C there is no change in the depression of excitability following the antidromic stimulus after injection of 2 mg strychnine per kg b wt which is a dose 10 times that claimed to block virtually all postsynaptic inhibition at the spinal level (Bradley *et al* 1953)

The possible presence of recurrent collaterals from the facial motor nerve fibers acting on the motoneurons was tested in another type of experiment as well A facial nerve branch was dissected into two parts one of which was used for delivering antidromic conditioning shocks and the other for eliciting an antidromic test response in the facial nucleus Regardless of the conditioning test interval no effects on the antidromic test response were seen These two types of experiment thus show that there are no recurrent collaterals from facial motor fibers with facilitating or inhibiting influences on the facial motoneurons Positive afterpotentials seem to be the major factor in the decreased motoneuron responsiveness after antidromic stimulation

Responses in the facial nucleus to stimulation of trigeminal afferents In response to electrical stimulation of a peripheral trigeminal nerve branch the type of potential illustrated in Fig 3 could be recorded in the brain stem at a site where maximal antidromic potentials were set up on facial nerve stimulation The response is a complex field potential A positive negative potential of very short latency and low amplitude (at arrow) is followed by a more prominent positive deflection of 2 5 ms duration This positive response merges into a negative response with a latency of 5 ms and a duration of more than 15 ms In the negative response there are two maxima one 7 5 ms and the other 15 ms after the peripheral stimulus The early positive negative deflection and the following positive potential were but little affected by an increase in stimulus frequency to 150/s The long lasting negative potential was hardly recordable at 10/s It is therefore reasonable to attribute the initial positive negative and the following positive potentials to the incoming volley in the spinal trigeminal tract perhaps also to activity in second-order trigeminal neurons (*cf* Wiesendanger *et al* 1967) The latencies of the two maxima of the negative potential correspond to those of the early and late components of the blink reflex corrected for efferent conduction time (Lindquist and Martensson 1970) Woody and Brozek (1969) have made an analysis of averaged potentials recorded in the facial nucleus with coarse stainless steel electrodes in response to a glabella tap They were not able to distinguish between the early and late component of the blink reflex

Excitability changes in the facial nucleus following afferent stimulation Using the antidromic test technique the excitability of facial motoneurons was also tested following tap stimulation of the face and electrical stimulation of the hypoglossal nerve afferents These experiments were undertaken to find out whether the conditioning effects on facial muscle reflexes previously obtained by these types of stimuli (Lindquist 1972) might to some extent be due to changes in motoneuron excitability Fig 4 is from such an experiment The plottings show the amplitudes of the conditioned antidromic responses at different conditioning test intervals as percentages of the calculated mean of unconditioned antidromic [illegible] trials It [illegible] pears that the motoneuron excitability is increased 3—3[illegible] after [illegible] stimulation and within this period two maxima occur The am[illegible]

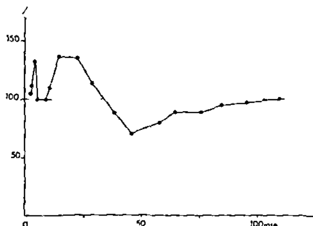


Fig 4 Amplitudes of antidromic responses in facial nucleus to stimulation of zygomatic branch following conditioning tap on facial skin as percentages of calculated mean of corresponding unconditioned potential amplitudes (ordinate) at different times after application of conditioning stimulus (abscissa)

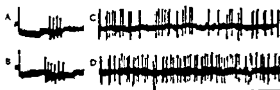
conditioned responses are 30—40 % larger than those of the unconditioned potentials probably due to antidromic invasion of motoneurons subliminally depolarized by the tap stimulus This facilitation is followed by a period of lowered motoneuron responsiveness setting in after a latency of 35 ms and lasting for 65 ms A minimum at which the response is only 70 % of the control value is recorded 45 ms following the stimulus The depressed responsiveness might be due to hyperpolarization of the motoneurons following a reflex activation but in spinal neurons orthodromic activation is not followed by long lasting positive afterpotentials as is antidromic activation (Frank and Fuortes 1955) Possible reasons why the antidromic responsiveness is reduced will be considered in the discussion

Similar effects were obtained when electrical excitation of the infraorbital or the frontal nerve was used as conditioning stimulus Antidromic responses to stimulation of the ventral facial branch were influenced by the afferent stimuli in a similar manner

Reflex activation of facial muscles may be obtained on stimulation of hypoglossal nerve afferents (Lindquist and Martensson 1969) In the present series of experiments excitability changes were observed in the facial motoneuron pool following stimulation of these afferents in curarized cats In one experiment antidromic invasion was facilitated for 25—30 ms after a latency of 13 ms Double shock stimulation was required to reveal the effects No depressed responsiveness was observed in these experiments possibly owing to absence of inhibitory effects from the hypoglossal afferents acting directly on the facial motoneurons Or else the antidromic test technique may not be capable of revealing more subtle postsynaptic processes

Properties of interneurons connected to facial nerve afferents The muscle branches of the facial nerve contain motor fibers and some afferents of unknown origin with excitatory as well as inhibitory influences on facial motoneurons (Lindquist and Martensson 1970) In the present series of microelectrode penetrations of the brain stem single neurons responding to stimulation of facial nerve branches with a

Fig 5 Discharges in single brain stem neuron in curarized cat *A* on stimulation of high threshold afferents in ventral branch of ipsilateral facial nerve *B* on stimulation of afferents in ipsilateral hypoglossal nerve *C* spontaneous activity which in *D* is increased by pinching the paw of the ipsilateral hind leg Time bars *A* and *B* 5 ms *C* and *D* 200 ms



threshold 6—10 times that of the motor fibers were often encountered The properties of 10 neurons were studied more closely in six different experiments

The recordings in Fig 5 *A*—*D* illustrate the activity in such a neuron in a curarized cat As shown in *A* the response to a single stimulus applied to the ventral branch of a *strength six times threshold for the motor fibers consists of 4 even spaced dis*charges of a frequency of 700/s and a latency of 11 ms Since also the hypoglossal nerve afferents have an inhibitory influence on facial muscle reflexes (Lindquist 1972) an experiment was made to see whether this neuron discharged also in response to afferent hypoglossal nerve stimulation The record in *B* illustrates the response of the neuron to a stimulus 10 times stronger than threshold for the hypoglossal motor fibers and as seen 6 repetitive discharges are elicited after 10 ms Since this neuron receives converging input from facial and hypoglossal afferents it may function as a link in an inhibitory pathway controlling facial muscle reflexes Also radial nerve afferents and afferents in the saphenous nerve have an inhibitory influence on facial muscle reflexes (Lindquist 1972) Hence the response of the neuron to stimulation of skin areas supplied by these nerves was also checked As shown in Fig 5 *C* the neuron discharged spontaneously at a mean frequency of 30/s On pinching the ipsilateral hind paw the discharge accelerated and reached a level of around 55/s as illustrated in *D*

The neuronal activity recorded in Fig 6 *A*—*D* is from the same experiment as the records in Fig 5 but the recordings were made 3 mm in the dorsocaudal direction from the site at which the maximal antidromic response to stimulation of the zygomatic branch was obtained In *A* *B* and *C* the zygomatic branch is excited with progressively stronger stimuli In *A* the stimulus is of threshold strength for eliciting a discharge in the neuron and approximately ten times threshold for the motor nerve fibers The single stimulus results after a latency of 15 ms in a synaptically relayed response consisting of 2 single discharges 1—2 ms apart In *B* the stimulus is stronger and the neuron responds after 10 ms with 3 successive discharges The maximal response to zygomatic nerve stimulation is shown in *C* in which a discharge of 7 impulses at 800/s is set up after 8 ms An increasing number of impulses at a successively higher discharge rate parallel to a decreasing response latency was a typical response of these neurons to increasing stimulus strengths A maximal discharge like that in *C* was abolished after a small number of stimulations at 4/s In *D* a maximal stimulation of the zygomatic branch is trailing a conditioning electrical

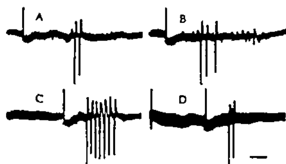


Fig 6 Discharges in single brain stem unit in response to progressively stronger electrical stimuli applied to ipsilateral zygomatic branch of facial nerve *A* threshold response *B* submaximal response *C* maximal response *D* only threshold response results when maximal stimulation of zygomatic branch is trailing a conditioning stimulus to ipsilateral infraorbital nerve Time bar 5 ms

stimulus to the infraorbital nerve by 17 ms The maximal discharge of 7 impulses illustrated in *C* is reduced to 2 impulses by the conditioning stimulus but the response latency is unchanged at 8 ms The neuron whose activity is illustrated in Fig 6 discharged also in response to tactile stimulation of hairs on the ipsilateral lower part of the face and the ipsilateral pinna Many of the neurons in this part of the brain stem that could be activated by stimulation of facial nerve afferents also responded to stimulation of the body and limbs Nociceptive stimuli were then usually required A few of these neurons also discharged on contralateral stimulation

Discussion

The antidromic potentials recorded in the facial nucleus do not differ markedly from antidromic potentials recorded extracellularly in other cranial motor nuclei (see *e g* Porter 1963 Morimoto *et al* 1968 Baker *et al* 1969)

In studies of spinal motoneurons the height of a monosynaptic test reflex has been widely used as a measure of excitability in spinal motoneuron pools This technique is however not applicable to studies of facial motoneurons since these obviously lack monosynaptic reflex connections (Lindquist and Mårtensson 1970) Instead amplitude variations of the potentials evoked in the facial motoneuron pool by antidromic stimulation have been used as an index of excitability The method is based on the well-documented fact that antidromic invasion of spinal motoneurons is facilitated by synaptic excitatory activity and depressed by synaptic inhibitory activity (for review see Eccles 1955) Reflex pathways to hypoglossal motoneurons have recently been analyzed in this manner (Morimoto *et al* 1968) When this method is used it is important to bear in mind certain limitations inherent in the procedure Thus out of the motoneurons contributing to an antidromic field potential some are totally invaded by the antidromic action potential whereas others may be blocked and thus give smaller contributions to the compound potential If all motoneurons are already totally invaded by the unconditioned antidromic stimulus it is not possible to demonstrate facilitation and conversely if all motoneurons are totally blocked it is not possible to demonstrate inhibition Furthermore block and release of block

occur in an all-or nothing manner and hence such effects are not to be expected from small synaptic potentials For the same reason the synaptic effects on anti dromic propagation probably have shorter durations than the synaptic potentials themselves since synaptic potentials show a peak and a following decay

The results obtained in this series of experiments suggest that inhibitory effects by recurrent collaterals from facial motor nerve fibers are lacking This is consistent with results from previous histological (Cajal 1952) and physiological studies (Lindquist and Martensson 1970) The lowered excitability in the motoneuron pool following antidromic invasion which sometimes lasted for 30 ms and on a few occasions even 100 ms is probably caused by the blocking effect of positive after potentials on the propagation in motoneurons (Eccles 1955) The duration of positive afterpotentials in lumbar motoneurons is usually longer than these values when measured with the monosynaptic test technique (Brooks and Wilson 1959) The duration of after hyperpolarization in spinal motoneurons may be correlated to the contraction times of the muscles innervated by them (Eccles *et al* 1958) If this applies to facial motoneurons and muscles then the results presented here indicate a wide range of contraction times for facial muscles with a mean lower than that for skeletal muscle in general Preliminary experiments suggest that this hypothesis is correct

The lowered excitability in the motoneuron pool following a tap stimulus to the face lends support to the idea that postsynaptic mechanisms are involved in the control of facial muscle reflex activity (Lindquist 1972) However the experimental method used does not allow a differentiation between disfacilitation and true post synaptic inhibition The duration of the inhibitory period recorded above indicates a repetitive inhibitory bombardment of the facial motoneurons It is however not long enough to account for the inhibitory periods of several hundred ms observed in previous experiments on facial reflexes (Lindquist 1972) The concept that pre synaptic inhibition may play a part in the control of facial muscle reflexes is thus not refuted

Afferents in the trigeminal hypoglossal facial and saphenous nerves have in common their inhibitory action on facial reflexes (Lindquist 1972 Lindquist and Martensson 1969 1970) In the last section of Results the properties of medullary neurons with converging input from all the e afferent sources were outlined These neurons—which are located in the lateral tegmental field (Berman 1968)—could be interneurons exerting an inhibitory control of facial reflexes Vyklicky *et al* (1967) seem to have recorded from neurons in the same general area even though they call it nucleus reticularis parvocellularis according to Meesen and Olszewski or nucleus reticularis ventralis according to Brodal The neurons studied by them gave repetitive responses to stimulation of peripheral trigeminal nerve branches and skin nerves in the ipsilateral foreleg The response latencies were 3—6 ms on stimulation of trigeminal branches and 11—12 ms on stimulation of other nerves The discharge of those neurons was also decreased by a conditioning trigeminal nerve volley Their location and the properties they have in common indicate that the

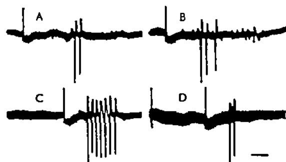


Fig 6 Discharges in single brain stem unit in response to progressively stronger electrical stimuli applied to ipsilateral zygomatic branch of facial nerve *A* threshold response *B* submaximal response *C* maximal response *D* only threshold response results when maximal stimulation of zygomatic branch is trailing a conditioning stimulus to ipsilateral infra orbital nerve Time bar 5 ms

stimulus to the infraorbital nerve by 17 ms The maximal discharge of 7 impulses illustrated in *C* is reduced to 2 impulses by the conditioning stimulus but the response latency is unchanged at 8 ms The neuron whose activity is illustrated in Fig 6 discharged also in response to tactile stimulation of hairs on the ipsilateral lower part of the face and the ipsilateral pinna Many of the neurons in this part of the brain stem that could be activated by stimulation of facial nerve afferents also responded to stimulation of the body and limbs Nociceptive stimuli were then usually required A few of these neurons also discharged on contralateral stimulation

Discussion

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In studies of spinal motoneurons the height of a monosynaptic test reflex has been widely used as a measure of excitability in spinal motoneuron pools This technique is however not applicable to studies of facial motoneurons since these obviously lack monosynaptic reflex connections (Lindquist and Mårtensson 1970) Instead amplitude variations of the potentials evoked in the facial motoneuron pool by antidromic stimulation have been used as an index of excitability The method is based on the well documented fact that antidromic invasion of spinal motoneurons is facilitated by synaptic excitatory activity and depressed by synaptic inhibitory activity (for review see Eccles 1955) Reflex pathways to hypoglossal motoneurons have recently been analyzed in this manner (Morimoto *et al* 1968) When this method is used it is important to bear in mind certain limitations inherent in the procedure Thus out of the motoneurons contributing to an antidromic field potential some are totally invaded by the antidromic action potential whereas others may be blocked and thus give smaller contributions to the compound potential If all motoneurons are already totally invaded by the unconditioned antidromic stimulus it is not possible to demonstrate facilitation and conversely if all motoneurons are totally blocked it is not possible to demonstrate inhibition Furthermore block and release of block

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Intracranial Pressure in Conscious Rabbits after Decentralization of the Superior Cervical Sympathetic Ganglia

By

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Abstract

Edvinsson L Ch Owman and K A West *Intracranial pressure in conscious rabbits after decentralization of the superior cervical sympathetic ganglia* Acta physiol scand 1972 85 193—200

The intracranial pressure measured as the ventricular fluid pressure was recorded continuously during about 2 days in conscious rabbits at various time periods after bilateral decentralization (preganglionic denervation) of the superior cervical sympathetic ganglia The pressure recorded 5 h after the operation was initially unchanged compared with non-denervated control animals During the following day it increased by approximately 50 mm physiological saline The pressure was elevated (by approximately 25 mm saline) also 6 days after the operation At 2 weeks it had returned to almost the same level as in the non-denervated controls. It is suggested that the alterations in the ventricular fluid pressure mainly reflect changes in the cerebral blood volume although direct effects on the sympathetically supplied choroid plexuses cannot be excluded

It was recently reported that bilateral removal of the superior cervical sympathetic ganglia markedly affected kaolin induced intracranial hypertension as revealed by short time measurements of the ventricular fluid pressure (VFP) in conscious rabbits (Owman and West 1970) The findings indicate that the sympathetic nervous system influences either or both of the two major factors maintaining and regulating the intracranial pressure namely the cerebral blood volume and the cerebrospinal fluid circulation

In order to obtain more detailed information about the mechanisms involved in this sympathetic influence a technique was devised for direct long term recording of the VFP in conscious rabbits (Edvinsson *et al* 1971 a) This technique revealed that the VFP varied markedly at different time periods after bilateral cervical sympathectomy (Edvinsson *et al* 1971 b) These variations were ascribed mainly to changes in the intracranial blood volume reflecting vasomotor reactions that could be expected to result from first leakage and subsequent disappearance of nor

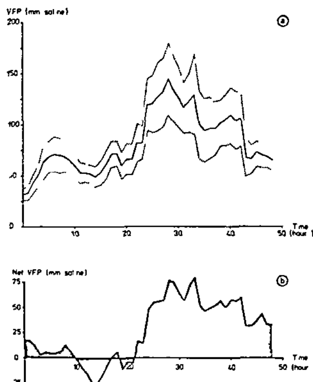


Fig. 1. (a) Mean VFP from recordings of animals 5 h after preganglionic denervation (decentralization) of the superior cervical sympathetic ganglia. Mean ——— ±SE. (b) Net VFP obtained by subtracting the value at each time interval in the methodological base curve of the control animals (Edvinsson *et al.* 1971 a) from the corresponding timerelated values in the curve of Fig. 1 a.

adrenaline from the sympathetic cerebro vascular nerve terminals (Nielsen and Owman 1967) followed by denervation supersensitivity of the vascular smooth muscles. This view was supported by direct studies on the alterations in cerebral blood volume in mice following cervical sympathectomy (Edvinsson *et al.* 1971 c). Impairment of the cerebrospinal fluid circulation by intracisternal kaolin injection was found to exaggerate the effects of sympathetic denervation on the VFP in rabbits (Edvinsson *et al.* 1971 d).

In attempts to investigate the importance of the central control of the superior cervical sympathetic ganglia with regard to the intracranial pressure, the VFP was recorded in conscious rabbits at various time periods after decentralization of the ganglia.

Materials and methods

Animals. The material consisted of 18 rabbits of either sex, weighing 2.3—3.0 kg. They were fed freely with standard pellets (SAN bolagen, Sweden), turnips and tap water *ad lib.*, also during the pressure recordings.

Pressure recordings. A small pressure cannula, occupying an intracranial volume of 0.0093 cm³ (Owman and West 1970), was inserted under local anesthesia into the left ventricle of the brain through a burr hole in the skull. The cannula was connected via a Statham Model P23AC pressure transducer to a Grass Model 7 Polygraph, and the VFP was continuously recorded in the conscious animals during about 2 days, as previously described in detail (Edvinsson *et al.* 1971 a).

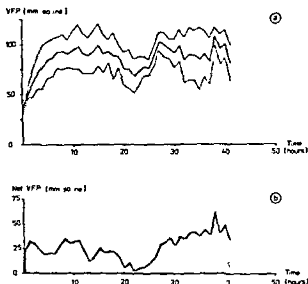


Fig 2 (a) Mean VFP from recordings on animals 6 days after decentralization Mean ——— ±SE (b) Corresponding net VFP (methodological base curve subtracted from curve in Fig 2 a)

Experimental groups Preganglionic denervation (decentralization) of the superior cervical sympathetic ganglia was performed by bilateral resection of about 2 cm of the sympathetic trunk from a level 3 mm belo the ganglion The operation was carried out through a small midline incision in the neck under dinitrous oxide halothane (Fluothane I C I) inhalation anesthesia The animals recovered within 10 min after the operation. The pressure cannula was inserted into the brain for subsequent recordings of the VFP at 5 h (Group 1 7 animals) 6 days (Group ? 4 animals) and 14 days (Group 3 7 animals) after the preganglionic denervation. Control animals see below

Pressure curves The pressure curves shown in Fig 1 a, 2 a and 3 a illustrate the measured *mean VFP* in each group of animals The initial value represents the mean pressure during the first 30 min of recording the remainder of the values shows the mean pressure during each subsequent 1 h period It has previously been shown (in non sympathectomized animals) that the implantation of the pressure cannula i i itself produces a period of increase in the VFP mainly due to the development of a local traumatic brain edema (Ed insson *et al* 19/1 a) The pressure curve obtained constitutes a "methodological base curve" and since the experiments were carried out during the course of the pre ent studies th is group of animals will serve as the control group In order to re eal the variations in the intracranial pressure specifically attributed to the preganglionic denervation each mean pressure value in the control groups was subtracted from the corresponding t me r lated mean pressure alues in each of Groups 1—3 The resulting values repre nt the *n t VFP* (the curves of which are demonstrated in Fig 1 b ?b and 3 b) and show the amount of p essure ariation in relation to the non sympathectomized control animals

Results

Group 1 VFP 5 h after denervation Recordings could be obtained from all 7 animals in this group up to 24 h One of the animals died at this stage and another animal at 34 h after insertion of the cannula Recordings was continued in the remaining 5 animals during totally 48 h The initial mean VFP was 31 mm physiological saline followed by an increase to about 60 mm saline during the subsequent 5 h (Fig 1 a) This level was essentially maintained for a further 15 h The mean VFP then increased fairly abruptly to more than 100 mm saline and the pressure

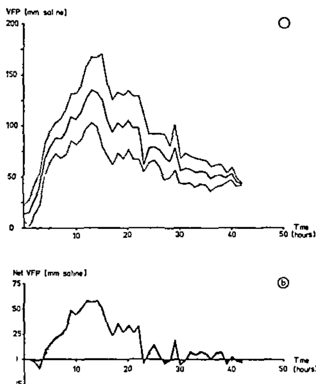


Fig 3 (a) Mean VFP from recordings on animals 14 days after decentralization Mean ——— ±SE (b) Corresponding net VFP (methodological base curve subtracted from curve in Fig 3 a)

remained at a high level during the remainder of the recording period It is probable that 2 animals in this group were lost because they did not survive this pronounced intracranial hypertension The curve illustrating the net VFP (Fig 1 b) did not reveal any greater difference in the intracranial pressure compared with non sympathectomized control animals during the first 20 h However the net VFP then increased rapidly to show high values between 35 and 80 mm saline during the remainder of the recording period thus demonstrating that the intracranial pressure had become significantly elevated in the denervated animals

Group 2 VFP 6 days after denervation *In this group all animals survived* and the recording system remained patent during the entire experiment lasting 41 h As seen in Fig 2 a the initial mean VFP was at 36 mm saline followed by a steady increase during the first 10 h to a level of about 90 mm saline The pressure was high during the rest of the recording period The net VFP calculated for this group of *animals (Fig 2 b) uniformly showed values above zero demonstrating that* the intracranial pressure was significantly increased as much as about 50 mm higher than in the control animals

Group 3 VFP 14 days after denervation After a recording period of about 24 h one of the 7 animals died and the measurements had to be discontinued in another

animal for technical reasons For the same reason they were discontinued in one animal also at 33 h The recording could be performed during a total of 42 h in the remaining 4 animals The mean VFP during the first 1/2 h was 14 mm saline (Fig 3 a) The mean VFP then increased within the following 12 h to 135 mm saline followed by a slower decrease to a level about 3 times higher than the initial level As shown in the net VFP curve (Fig 3 b) the intracranial pressure in the denervated animals was at the same level as in the controls during the first 4 h and the last 19 h of the recording period In between the pressure was markedly elevated up to 60 mm saline in the denervated animals

Discussion

The intracranial pressure measured as the ventricular fluid pressure (VFP) was recorded continuously in conscious rabbits during 41—48 h at various time periods after bilateral preganglionic denervation (decentralization) of the superior cervical sympathetic ganglia During degeneration the preganglionic nerves are known to lose their ability to synthesize acetylcholine within 3 days and the content of the acetylcholine transmittor is markedly reduced (MacIntosh 1938, Feldberg 1943) This coincides with the impairment and subsequent abolishment of synaptic transmission in the sympathetic ganglia (Coppee and Bacq 1938 MacIntosh 1938) In the ganglion cells the decentralization results in a rise in the noradrenaline level which is probably due to the lack of physiological stimulation after deprivation of the afferent innervation (see Giacobini 1970) This may account for the tendency to an increase in the noradrenaline transmittor found also in the nerve fibres of sympathetically innervated pial vessels after decentralization (Edvinsson *et al* 1972 a)

It has previously been shown that bilateral superior cervical sympathectomy (postganglionic denervation) brings about significant alterations in the VFP (Edvinsson *et al* 1971 b and d) The alterations were interpreted as the result mainly of those changes that can be expected to occur in the cerebral blood volume after sympathectomy (*cf* Edvinsson *et al* 1971 c) In addition it is possible that compensating secondary phenomena such as development of alternate pathways for cerebrospinal fluid absorption (Sahar *et al* 1969 1970) and a reduction in its formation (Calhoun *et al* 1967 Hochwald *et al* 1969 Lorenzo *et al* 1970) were involved when the VFP became high Since not only the pial vessels (Nielsen and Owman 1967) but also the choroid plexuses (Edvinsson *et al* 1972 b) receive an adrenergic nerve supply it cannot be excluded that the interference with the cervical sympathetic system also induced direct changes in the cerebrospinal fluid formation (Edvinsson *et al* 1972 c) Moreover it has recently been found that the extent of induced brain edema is influenced by the cervical sympathetic nervous system (West 1971) However it is conceivable that these latter changes are secondary to alterations in one or both of the two previously discussed mechanisms the cerebral blood circulation and the cerebrospinal fluid dynamics

The direct pressure recording technique used involves the implantation of a small cannula into the lateral ventricle of the brain a procedure which in itself has been found to produce an increase in the VFP due primarily to a local traumatic brain edema (Edvinsson *et al* 1971 a) In order to evaluate any specific effect of decentralization on the VFP, the pressure values in these control animals were subtracted from the corresponding values obtained in the present series of denervated animals When measured 5 h after decentralization of the superior cervical sympathetic ganglia (*i e* sectioning of the preganglionic cholinergic nerves) the VFP was initially found to be at a level similar to that in the non denervated control animals This was followed a day later by an increase in the VFP to a fairly high and constant level It is known that initially after severing cholinergic autonomic nerves there is a transient activation of the effector structure (Emmelin and Stromblad 1957 Emmelin 1968) probably as a consequence of transmitter leakage (MacIntosh 1938, Feldberg 1943) from the degenerating nerve terminals With regard to the superior cervical ganglia this activation may account for the initially low net VFP When the transmitter has subsequently disappeared from the preganglionic nerve terminals the activation subsides and the synaptic transmission becomes impaired and is then abolished (Coppee and Bacq 1938 MacIntosh 1938) Owing to the lack of physiologic stimulation of the superior cervical sympathetic ganglia after deprivation of their afferent nerve supply the transmitter release from the postganglionic sympathetic nerves is probably reduced as evidenced by an increased level of noradrenaline in the ganglion cell bodies (see Giacobini 1970) and in the sympathetically innervated pial vessels (Edvinsson *et al* 1972 a) At this stage the cerebral blood volume has been found to be increased (Edvinsson *et al* 1972 d) conceivably due to a cerebral vasodilation This would explain the relatively high net VFP which was evident already during the second day (Group 1) but also a week (Group 2) after decentralization However it can be assumed that the cerebral vasodilation (and thus the increase in the VFP) to a certain extent has been counteracted by the slight decentralization supersensitivity of the sympathetically innervated brain vessels that can be expected to have developed at the one week stage (Langer *et al* 1967)

2 weeks after postganglionic denervation the effector structure has achieved a very high degree of supersensitivity to circulating catecholamines (Langer *et al* 1967) Such a supersensitivity was believed to result in a normal—or even higher—tone in the denervated brain vessels at this stage after ganglionectomy which consequently would explain the normal—or even subnormal—VFP recorded (Edvinsson *et al* 1971 b) Further it was assumed that a stress induced increase in circulating catecholamines in connection with the implantation of the pressure cannula produced a transient intracranial vasospasm in the supersensitive denervated vessels A hypoxic brain edema following this circulatory disturbance was suggested to explain the subsequent short lasting increase in VFP recorded after the implantation (Edvinsson *et al* 1971 b) In addition it has recently been found that an experimentally induced brain edema becomes more pronounced if cervical sympathetic denervation has been performed 2 weeks before (West 1972)

2 weeks after decentralization (preganglionic denervation) on the other hand it is known that the supersensitivity is considerable less pronounced than after post ganglionic denervation (Langer *et al* 1967) It has also been shown that at this post operative stage the cerebrospinal fluid formation in the choroid plexus is probably decreased in ganglionectomized rabbits (Edvinsson *et al* 1972 c) These differences may explain the tendency to higher VFP in the decentralized (Group 3) compared with the corresponding ganglionectomized (Edvinsson *et al* 1971 b) rabbits although the pressure patterns otherwise resemble each other in the 2 experimental groups

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Concentration of Noradrenaline in Pial Vessels, Choroid Plexus, and Iris during Two Weeks after Sympathetic Ganglionectomy or Decentralization

By

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Abstract

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Changes in the noradrenaline concentration of the sympathetically innervated pial vessels choroid plexuses and irides of the rabbit were measured fluorometrically at various periods during the first 2 weeks after pre or postganglionic denervation (decentralization or excision of the superior cervical ganglia) The noradrenaline values fell to negligible amounts within 2 days after ganglionectomy in all organs probably somewhat slower in choroid plexuses After decentralization the noradrenaline level increased in the pial vessels whereas in the choroid plexuses and iris it initially decreased followed by normalization The fall occurred somewhat later in the iris. It is suggested that the results of the denervation experiments reflects the presence of functionally different types of sympathetic neurons in one and the same ganglion

It has been accepted since long that postganglionic denervation of sympathetically innervated organs is accompanied by a disappearance of noradrenaline from the organ (Euler and Purkhold 1951 Goodall 1951) whereas preganglionic denervation has little or no effect on the tissue concentration of the noradrealine transmitter (Rehn 1958) Since basic studies on the neural control of structures with a sympathetic supply usually involve surgical interference with the nerves it is necessary to obtain detailed information about the time course of changes in the transmitter concentration after the denervation Fluorescence histochemistry has revealed that the pial circulation (Falck *et al* 1965 1968 Nielsen and Owman 1967 Spoendlin and Lichtensteiger 1967 Donáth 1968 Kajikawa 1968 Ohgushi 1968) and the choroid plexus (Edvinsson *et al* 1972 b) are provided with sympathetic nerves which arise in the superior cervical ganglia and which may influence brain circulation (Edvinsson *et al* 1971 a Kobayashi *et al* 1971 Nielsen *et al* 1971) cerebro-

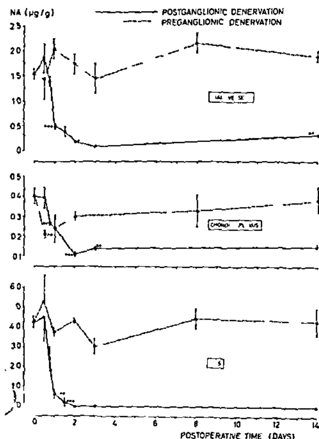


Fig 1 Organ concentration of noradrenaline ($\mu g/g$ wet weight) at various periods after post ganglionic denervation (bilateral excision of the superior cervical sympathetic ganglia) or pre ganglionic denervation (decen tralization) Organs from 3 ani mals were pooled for each deter mination Each point gives the mean ($\pm$S E) of 4 determina tions (pial vessels time 0 10 determinations) Differences be tween non operated (post-oper ative time 0 days) and oper ated animals *0 01 < P < 0 05 **0 001 < P < 0 01 and ***P < 0 001

spinal fluid formation (Edvinsson *et al* 1972 a) and intracranial pressure (Ow man and West 1970 Edvinsson *et al* 1971 b)

The present report describes the detailed pattern of fluorometrically determined changes in the noradrenaline concentration in rabbit pial vessels and choroid plexus during the first 2 weeks after excision or decentralization of the superior cervical sympathetic ganglia The sympathetic innervation of the iris which is a well studied model organ was used for comparison

Material and methods

The material comprised 219 albinic and randomly pigmented rabbits of either sex weighing 2—3 kg They were maintained on standard pellet food (SAN bolagen Sweden) turnips carrots and tap water *ad lib*

The operations were performed via a ventral midline incision in the neck with the animals in dinitrous oxide halothane (Fluothan I C I) anesthesia After suturing the wound the animals recovered within about 30 min Three types of operations were performed Post ganglionic denervation (84 animals) bilateral extirpation of the superior cervical ganglia together with approximately 5 mm of the sympathetic trunk above and below the ganglion The animals were killed at various time periods after the operation (Fig 1) Preganglionic denerva tion (72 animals) bilateral excision of 20 mm of the sympathetic trunk from 3 mm below the

superior cervical ganglion The time intervals for sacrifice of the animals is seen in Fig 1 Sham-operation (12 animals) all steps in the above operations were performed except that the sympathetics were left intact These animals were killed after 12 h to check any effects of anesthesia and the immediate operative trauma 30 non-operated animals served as untreated controls

All animals were killed by i v air injection between 8 a.m. and 3 p m. The entire iris on both sides (total wet weight approximately 0 5 g) and the choroid plexuses from the lateral and fourth ventricles (total wet weight approximately 60 mg) were dissected out. The pia arachnoid including the pial vessels (total tissue wet weight approximately 25 mg) was freed from the base of the brain care being taken to avoid contamination with brain tissue The tissues were homogenized in ice-cold 0 4 N perchloric acid and noradrenaline was extracted and determined fluorometrically according to the method of Bertler *et al* (1958) as modified by Häggendal (1963) Tissues from 3 animals were pooled for each determination Each time interval with each denervation procedure in Fig 1 shows the mean of 4 determinations (on 12 animals) except for the value from pial vessels at time 0 which is based on 10 determinations (30 animals)

Statistical differences between mean values were calculated according to Student s *t* test

The effect of the denervation procedures was checked by fluorescence microscopy of noradrenaline (for methodological principles see Falck 1962 Falck *et al* 1962 Corrodi and Jonsson 1967) in the above mentioned 3 tissues from an additional 21 animals 5 untreated controls 4 with postganglionic and 4 with preganglionic denervation killed 8 days later and another 4 plus 4 animals killed after 14 days The tissues were immediately dissected out frozen to the temperature of liquid nitrogen freeze-dried treated for 1 h at 80 C in formaldehyde gas embedded in paraffin and serially sectioned at 6 μ thickness for fluorescence microscopy (for technical details see Falck and Owman 1965) The reliability in evaluting changes in fluorescence intensity was secured by reading the slides on a blind basis without knowing which operative treatment the animals had received

Results

The pial vascular preparation contained 1 50 μg/g noradrenaline the choroid plexuses 0 40 μg/g and iris 4 32 μg/g in normal untreated animals The changes in the tissue concentration of the amine after bilateral superior cervical ganglionectomy (postganglionic denervation) or excision of the cervical sympathetic trunks (preganglionic denervation) are illustrated in Fig 1 The noradrenaline concentration in the 3 tissues from the sham-operated animals did not differ significantly from that of either the untreated controls or the denervated controls at the corresponding time period (12 h postoperatively) After postganglionic denervation a significant reduction in noradrenaline could not be registered until at or after 18 h postoperatively and at 48 h noradrenaline had disappeared from all tissues *i e* the amine values measured at this and the following time intervals were insignificant

The presence of adrenergic nerve terminals around the main pial arteries in the iris dilator and sphincter muscles and in the choroid plexuses confirmed earlier findings (Ehinger 1964 Falck *et al* 1965 Edvinsson *et al* 1972 b) and agreed with weeks after ganglionectomy the noradrenaline fluorescence was abolished from all adrenergic nerves

After preganglionic denervation little or no changes were noted quantitatively in the noradrenaline of the pial vascular preparation during the initial postoperative days (Fig 1) However after 1 and 2 weeks the concentration had increased by 40 and 20 per cent respectively The statistical significance of this increment was supported by the finding that the fluorescence intensity in the pial vascular nerves was slightly but definitely higher in the 1 and 2 week animals compared to the

untreated controls In the iris the noradrenaline level remained essentially unchanged during the entire experimental period (Fig 1) in accordance with an unaltered fluorescence intensity though a probably significant reduction occurred 3 days postoperatively In the choroid plexus on the other hand the noradrenaline concentration showed a significant about 50 per cent reduction 12 h after the decentralization and the concentration was still reduced by approximately 25 per cent compared with the untreated controls after 48 h In the remainder of the experimental period the level was not significantly lower than in the controls although it tended to be subnormal The fluorescence intensity of the sympathetic nerves in the choroid plexuses did not seem to differ from that of the non operated animals at any time interval

Discussion

The time course of disappearance of fluorometrically determined noradrenaline in the rabbit iris after postganglionic denervation (superior cervical ganglionectomy) agrees with observations presented for other sympathetically innervated organs in different animal species (Euler and Purkhold 1951 Goodall 1951 Furchgott 1960 Sidman *et al* 1962 Weiner *et al* 1962 Benmiloud and Euler 1963 Smith *et al* 1966 Sedvall 1969) The findings are well correlated with the disappearance of formaldehyde induced noradrenaline fluorescence from the postganglionic adrenergic adrenergic nerves after the operation (Falck 1962 Malmfors and Sachs 1965 Falck and Owman 1966 Van Orden *et al* 1967) Using the fluorescence technique Kajikawa (1968) recorded an abolished fluorescence in the adrenergic nerves of the middle cerebral artery of guinea pigs and rats 5 days after superior cervical ganglionectomy This is a somewhat later disappearance of the transmitter than presently observed chemically in the rabbit pial vessels It may be that the fluorescence technique is a more sensitive means of detecting residual noradrenaline in scattered nerve terminals or the discrepancy may be due to a species difference

Histochemical and chemical studies following administration of noradrenaline have shown that the uptake and retention of the amine operate shortly after ganglionectomy but is then deteriorated with approximately the same speed as the endogenous transmitter disapperance (Malmfors and Sachs 1965 Sears and Gillis 1967 Sedvall 1969) Concomitantly there is an increase in the release of noradrenaline from the nerves (Sears and Gillis 1967) which explains the activation of the adrenergic receptors (see Lundberg 1970) during the first phase of the nerve degeneration A slower loss of noradrenaline from the choroid plexus tissue after postganglionic denervation could explain the measured tendency to a delay in noradrenaline reduction compared with that found for the pial vessels and iris

On the basis of previous chemical and histochemical studies it is generally accepted that preganglionic sympathetic denervation (decentralization) does not alter *the organ content of noradrenaline* (Rehn 1958 Falck 1962) From the present figures it can be seen however that a generalization of the phenomenon

is not possible to make the results obviously depend on the organ studied and at which time after denervation the analysis is performed Thus in the choroid plexus a 50 per cent reduction in the noradrenaline concentration was registered 12 h after the decentralization followed by a slow normalization of the amine level In the iris a similar tendency was seen although the reduction occurred 2 days later In the pial vessels on the other hand the transmitter concentration showed a post operative increase so that a week after decentralization the amine level was 40 per cent higher than in non-operated controls Also the results on the noradrenaline content of the postsynaptic ganglion cell bodies after the decentralization proce dure are inconsistent although the observations seem to favour an increase in ganglionic noradrenaline after the operation (see Giacobini 1970)

The noradrenaline level that can be registered in the postsynaptic sympathetic neuron after deprivation of its afferent innervation appears to be a function of two counteracting mechanisms in the neuron—a diminished physiological activity and a lowered noradrenaline synthesis (Sedvall 1969) The net neuronal concentration of noradrenaline in a given neuron after decentralization will therefore depend on the relative alteration in its activity (rate of noradrenaline release) and rate of noradrenaline formation It is possible that the observed variation in net noradrena line after decentralization reflects a different rate in the functional turn-over of the transmitter which would mean that different types of sympathetic neurons are present in one and the same ganglion

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Acta physiol scand 1972 85 207—211

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Hemodynamic Responses of Relaxed and Shivering Hypothermic New-Born Lambs

By

ANTTI KOIVIKKO and ESKO LÄNSIMIES

Received 12 November 1971

Abstract

KOIVIKKO A and E LÄNSIMIES *Hemodynamic responses of relaxed and shivering hypothermic new born lambs* Acta physiol scand 1972 *85* 207—211

The hemodynamic responses to hypothermia and shivering were investigated in 4 relaxed in 9 deeply anesthetized and 9 lightly anesthetized new born lambs. Muscle relaxation and deep anesthesia prevented the increases of the oxygen consumption and the cardiac output In shivering lambs these parameters increased Correction of metabolic acidosis increased temporarily the cardiac outputs The cardiac responses to cold stress of the relaxed and deeply anesthetized lambs differed from that seen in the relaxed adult dogs but were similar to those observed in relaxed β blocked dogs This difference may be due to some kind of immaturity of the β adrenergic system in the new born period

Recent clinical and experimental results show that hypothermia can protect new born infants (Cordey 1964 Miller Miller and Westin 1964 Westin *et al* 1962) and other newborn mammals (Zakhary Miller and Miller 1967) from the influence of severe asphyxia Exposure to cold has also been found to have adverse effects on premature and full term infants as it increases the oxygen consumption instead of decreasing it (Adams Fujiwara and Spears 1954 Adamsons Gandy and James 1965 Brück 1961) There are few hemodynamic studies about hypothermia in the neonatal period. Koivikko (1970) has investigated the cardiovascular response of neonatal lambs in a recent study The aim of the present study was to clarify some aspects found then namely the influence of metabolic acidosis on the cardiac response during shivering in hypothermia A similar study on adult dogs was also carried out to compare the hemodynamic responses to shivering in the neonatal period to those in adult mammals (Halkola Koivikko and Lansimies 1972)

Material and Methods

The animal material comprised 22 lambs 1—[illegible] days old 9 of them were anesthetized with chloralose to prevent shivering 4 animals were relaxed with pancuronium bromide (Pavulon® Organon Netherlands) to prevent all spontaneous muscular activity 9 animals were given the anesthetic for sedation only and were allowed to shiver All the animals were ventilated

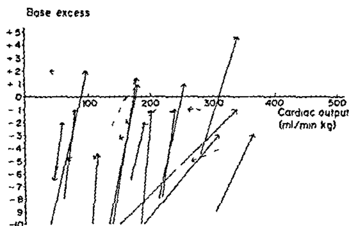


Fig 5 The effects of sodium bicarbonate injections upon the cardiac output and base excess of the hypothermic new born lambs (→) The new determinations 2—3 min later (—→) are shown in some cases

In this serie and other lambs studied in hypothermia disturbances of cardiac rhythm have not been observed

Discussion

Shivering during hypothermia proved to be a potent heat producing system This study must be extended to draw conclusions about the non shivering heat production observed in newborn guinea pigs rabbits and human infants (Bruck 1961)

Although our results of the influence of metabolic acidosis on the cardiac response during shivering are not entirely clear cut it can be concluded that metabolic acidosis may suppress the increase of the cardiac output during shivering This was probably the reason for the earlier finding of poor cardiac response to increased oxygen consumption during shivering (Koivikko 1970) In the present series of lambs those with normal or nearly normal bicarbonate levels in the arterial blood were able to increase cardiac outputs when the oxygen consumption increased during shivering

The role of metabolic acidosis in preventing cardiac responses of beta adrenergic type remained obscure because of the overlap between results from acidotic and normal lambs The correction of B E by infusions of sodium bicarbonate had only a temporary effect on the cardiac output

Although the initial normothermic level of cardiac output is much higher than that of adult dogs (Halkola et al 1972) the relative increase was nearly the same The largest increases were up to 600 to 700 ml/min kg b w when the oxygen consumption increased to 40—45 ml/min kg b w This indicates that also newborn lambs have cardiac reserve This was achieved in these experimental conditions by increments in the stroke volume They increased to higher values than that of adult dogs On the other hand heart rates were not influenced by different experimental conditions in either lambs or dogs

There is an interesting difference between the cardiac output responses of relaxed lambs and dogs In relaxed dogs although the oxygen consumption during cooling decreased the cardiac outputs remained at a high level In contrast the cardiac outputs of relaxed lambs and relaxed β blocked dogs decreased These results indicate that the cold stress alone has some β stimulating effect upon the heart of adult dog but not on that of the neonatal lambs It remains to be solved whether the epinephrine release due to cold stress or the β adrenergic cardiac response of the neonatal lambs is still immature The β adrenergic activity seems to be present in the hearts of neonatal lambs because increases of cardiac output due to hypoxia hypercapnia and epinephrine infusions may be prevented by propanolol (Koivikko unpublished data) On the other hand the adrenal glands of newborn lambs are shown to excrete catechols as well as those of adult sheep in response to asphyxia (Comline and Silver 1966)

This study was supported by a grant from Yrjo Jahnssonin Saatio

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Acta physiol scand 1972 85 212—216

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Hemodynamic Responses of Relaxed, β-Blocked and Shivering Dogs during Hypothermia

By

LAURI HALKOLA, ANTTI KOIVIKKO and ESKO LÄNSIMIES

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Abstract

HALKOLA L A KOIVIKKO and E LÄNSIMIES *Hemodynamic responses of relaxed β blocked and shivering dogs during hypothermia* Acta physiol scand 1972 *85* 212—216

7 dogs were anesthetized only ten dogs anesthetized and relaxed Additionaly three dogs were anesthetized relaxed and β blocked All dogs were ventilated by oxygen Hypothermia was produced by ice water immersion The oxygen consumption decreased in all but the shivering group Initially the cardiac output increased in the shivering group decreased in the β blocked group but an unexpected slight increase was observed in the relaxed group The reason for this observation is being discussed

Previous studies of cardiorespiratory responses of adult dogs to hypothermia have revealed increased oxygen consumption and cardiac output in animals shivering in tact and decreased values of these parameters in relaxed dogs (Prec *et al* 1949 Westin Sehgal and Assali 1961 Thauer 1965 Gribbe *et al* 1961) This study was carried out as a control series of a similar study on newborn lambs (Koivikko and Lansimies 1972)

Material and Methods

20 dogs were anesthetized with sodium pentobarbital (Nembutal® Abbot 25—30 mg/kg b w) The dogs were intubated and connected with Starlings ideal pump Temperature was recorded with oesophageal electrode (Electrolaboratoriet Denmark)

Hypothermia was produced by ice water immersion 7 dogs were only given the anesthetic agent 10 dogs were relaxed by pancuronium bromide (Pavulon® Organon Holland 50 μg/kg b w) Additionally 3 dogs were given also propranolol (Inderal® I C I 0 5 mg/kg) for β blockade 30 min before the cooling period

Aortic and pulmonary artery pressures and ECG were recorded (Elema, Sweden) Cardiac output and stroke volume were determined by dye-dilution method (Atlas Cardiognost West Germany) Cardio-Green® was used as indicator Arterial blood samples were drawn for Po Pco and pH determination at 37 C (IL 213—227 Italy) The values were corrected to the actual body temperature of the dog (Kelman and Nunn 1966) When metabolic acidosis was observed it was corrected with infusions of 7 5 % sodium bicarbonate (Leiras Finland) Oxygen consumption was measured with Krogh s spirometer The registrations were made at intervals of 1 C

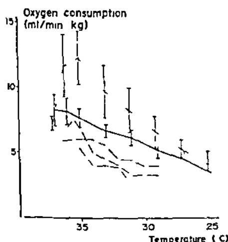


Fig 1

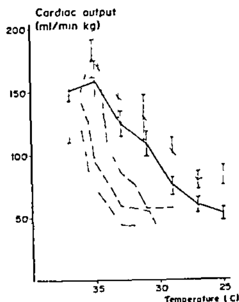


Fig 2

Fig 1 Oxygen consumptions in shivering (— — — —) relaxed (————) and relaxed β blocked (— — —) dogs during hypothermia Means and standard errors of the means in the two former groups are shown

Fig 2 Cardiac outputs in shivering relaxed and relaxed β blocked hypothermic dogs Symbols as in Fig 1

Results

The oxygen consumption of the relaxed non shivering dogs decreased when temperature decreased (Fig 1) In shivering dogs the mean oxygen consumption increased by about 50 % at oesophageal temperature of 35° C ($p < 0\ 05$) Below this temperature the oxygen consumption of the shivering dogs gradually decreased approaching the values of the non shivering animals The maximum oxygen uptake observed was 20 ml min kg

TABLE I Acid base balance (Means in each group)

I relaxed
II shivering
III β blocked r l x d dogs

	37—35 C			34—33 C			32—31 C		
	I	II	III	I	II	III	I	II	III
pH	7 57	7 39	7 68	7 63	7 25	7 78	7 66	7.28	7 78
P_{CO}	26	46	14	18	51	10	16	50	8
P_{O}	189	—	330	223	—	334	235	—	227
BE	—2 8	+0 3	—1 2	—2 5	—3.2	—0 3	—2 3	—4 5	—1 8

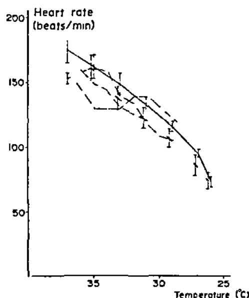


Fig 3

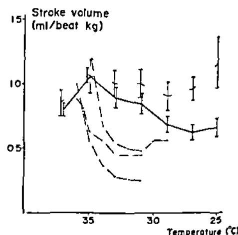


Fig 4

Fig 3 Heart rates of the shivering relaxed and relaxed β blocked hypothermic dogs Symbols as in Fig 1

Fig 4 Stroke volumes of the shivering relaxed and relaxed β blocked hypothermic dogs Symbols as in Fig 1

The relaxed respirator ventilated dogs developed a respiratory alkalosis (Table I) whereas the shivering dogs showed a slight tendency towards increased values of P_{CO} In the β blocked group P_{CO_2} values were slightly lower than those of the relaxed dogs According to the PaO values the dogs were not hypoxic

The cardiac output (Fig 2) in the relaxed dogs tended to remain at the pre experimental level to 35° C After this the cardiac output decreased to 1/3 of the initial level when the temperature decreased to 25° C In the shivering dogs the cardiac output increased at the beginning of the immersion by 40 per cent ($p <$ 0 001) of the initial value and the slope of decrease parallelled to that of the relaxed

30—39 C			28—27 C			26—?5 C		
I	II	III	I	II	III	I	II	III
7 60	7 35	7 76	7 72	7 27		7 63	7 11	
21	44	9	13	59		17	93	
2??	—	219	2?5	—		213	—	
+1 0	—? 5	—1 5	0	—1 5		—1 0	—4 5	

dogs The cardiac outputs were generally higher in the shivering dogs but there was no statistically significant difference between these groups

The initial increase of cardiac output at the beginning of hypothermia was due to increases of both heart rate (Fig 3) and stroke volume in shivering animals (Fig 4) Only the stroke volume increased in the relaxed dogs at the beginning of hypothermia Below the temperature of 35° C the heart rates in both groups decreased nearly equally In this temperature range the stroke volume of the shivering dogs remained at the increased level On the contrary the stroke volume of the relaxed dogs decreased

The cardiac outputs of β blocked relaxed dogs decreased already at the beginning of the cooling in contrast to the relaxed dogs This was due to a decrease of the stroke volume The oxygen consumption of these dogs was less than that of relaxed dogs Otherwise there were no marked differences in the measured parameters

Discussion

Comparisons between different studies concerning hypothermia may be misleading because of differences in anesthesia relaxation respiration etc In this study the differences between the three experimental groups of dogs were the following the relaxed ones were given pancuronium bromide and the blocked group propranolol + pancuronium Earlier studies have shown that administration of pancuronium bromide suppresses the cardiac output only slightly (Lansimies Klossner and Hirvonen 1971) Positive pressure respiration may affect the cardiorespiratory functions for example oxygen consumption may increase (Cain 1970) Cardiac output heart rate and stroke volume may be influenced by continuous positive pressure breathing (Salzano and Hall 1965) The respiratory alkalosis of the relaxed dogs *vs* the increase of P_{CO} of the shivering dogs may have changed the reactions during cooling (Carson *et al* 1965) although before the cooling period the differences of the parameters measured were insignificant

The first stage of cooling produced an increase of the oxygen consumption in the shivering animals and a decrease of that in the relaxed dogs Both these changes were expected The lowering of the oxygen consumption in the dogs given propranolol may reflect the suppression of the cathecol induced thermogenesis observed in rabbits (Hull 1963) The cardiac output of the shivering dogs increased (Prec *et al* 1959) and corresponds to the thesis that there is a linear relationship between cardiac output and oxygen consumption during hypothermia (Thauer 1965) On the other hand the cardiac output of the relaxed dogs did not follow this thesis It remained at the initial level during the first 10 to 15 min of hypothermia down to 35° C This response was not observed by Prec *et al* (1949) nor by Westin *et al* (1961) The difference of their results may be due to the sodium pentobarbital given by these investigators enough to suppress shivering This drug may have suppressed the adrenergic stimuli on the heart (Greisheimer 1965) In our series the cardiac output may have been high because of cathecolamines excreted during co

stress which is shown by LeBlanc *et al* (1961) and Leduc (1961) The effect of the β blockade on the cardiac output in the three relaxed dogs shows that this may hold true Cold acclimatized dogs have somewhat similar responses of cardiac function to those of relaxed dogs (Evonuk 1966) However our dogs were likely to be warm acclimatized because most of them had been grown inside and some had made only short visits outside Anyway this aspect is very important when selecting animals to hypothermia studies

Below 35° C the cardiorespiratory parameters varied as expected in the shivering animals The cardiac outputs of the shivering and relaxed groups did not differ significantly This reflects possibly further the influence of catechols on the heart in the relaxed group because in β blocked dogs the cardiac outputs were significantly lower

Aortic pressures of the shivering and relaxed dogs differed significantly first below 30° C Rough calculations show that the peripheral flow resistance increased slightly in both groups which agrees with the earlier findings (Westin *et al* 1961)

This study was supported by a grant from Yrjo Jahnssonin Saatio

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Acta physiol scand 1972 85 217—227

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Characteristics of Monosaccharide Permeability in Arterial Tissue and Intestinal Smooth Muscle, Effect of Insulin

By

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Received 12 November 1971

Abstract

ARNQVIST H J *Characteristics of monosaccharide permeability in arterial tissue and intestinal smooth muscles effect of insulin* Acta physiol scand 1972 *85* 217—227

Monosaccharide permeability was studied in bovine mesenteric arteries and rabbit intestinal smooth muscle ^{14}C labelled 3 0-methylglucose was used to study counter transport and saturation kinetics L-glucose 1 ^{14}C and D glucose 1 ^{14}C were used to study substrate stereospecificity ^{14}C and ^{3}H labelled sorbitol were used to estimate the extracellular space In both bovine mesenteric arteries and smooth muscle from rabbit colon the membrane permeability of monosaccharides was characterized by substrate stereospecificity counter transport and saturation kinetics Monosaccharides were transported down a concentration gradient These results suggest that monosaccharides penetrate vascular and intestinal smooth muscle cell membranes by facilitated diffusion In the concentration range 5 6—44 4 mM the distribution of unlabelled glucose in bovine mesenteric arteries corresponded to the distribution of sorbitol ^{14}C indicating that the membrane transport of glucose was rate limiting for the glucose metabolism Insulin (0 1 U/ml) increased to a small degree the membrane transport of 3-0-methylglucose in bovine mesenteric arteries Compared with the effect of insulin on monosaccharide transport in skeletal muscle the effects on monosaccharide transport in bovine mesenteric arteries and rabbit colon smooth muscle were very small

Factors affecting the metabolism of arterial tissue may be of importance in the development of vascular disorders (Kirk 1968 Whereat 1967) As patients with diabetes mellitus show an increased frequency of vascular diseases (Schettler and Wahl 1969) it is of great interest to know how the diabetic state influences the metabolism of arterial tissue In an earlier work (Arnqvist 1971) it was found that insulin (0 1 U/ml) moderately stimulated the accumulation of ^{14}C labelled glucose in rat aorta and smooth muscle from rabbit colon The distribution of D xylose ^{14}C in rabbit colon was increased to a small degree by insulin indicating an effect on the smooth muscle cell membrane It was also shown that the cell membrane had a rate limiting effect on the glucose metabolism in rabbit colon

In skeletal muscle and adipose tissue monosaccharide transport systems have been identified which exhibit substrate stereospecificity counter transport and satura-

kinetics (Morgan Regan and Park 1964 Crofford and Renold 1965) As the monosaccharides are transported down a concentration gradient this type of transport is called facilitated diffusion (Danielli 1954) The transport of glucose *across the cell membrane is in both tissues an important rate limiting step in the* glucose uptake and plays a part in metabolic control

The aim of the present investigation was to determine whether a specific membrane transport system exists for monosaccharides in arterial tissue and smooth muscle and to elucidate the characteristics of the membrane permeability of monosaccharides ^{14}C labelled 3 0 methylglucose a monosaccharide that closely resembles glucose in its transport across the cell membrane (Narahara and Özand 1963) and that is not metabolized by these tissues (see Methods) was selected to study saturation kinetics and counter transport The tissue accumulation of glucose carbon from ^{14}C labelled D and L-glucose was studied to find out whether the membrane permeability showed substrate stereospecificity for monosaccharides The tissue distribution of unlabelled glucose at varying concentrations was determined by an enzymatic method and compared with the extracellular space to see if the membrane transport of monosaccharides was rate limiting for the glucose metabolism.

Material and Methods

Animals Rabbits (2—3 kg) and male rats (60—100 g) of the Sprague Dawley strain were used Before the experiments all animals were starved for 20—24 h to reduce their endogenous insulin secretion Bovine mesenteric arteries were obtained from a slaughter house

Dissection and incubation The muscle layer of rabbit colon was dissected as previously described (Arnqvist 1971) Bovine mesenteric arteries were dissected out approximately 30 min after slaughter A homogenous 10—15 cm long segment was cut off and transported to the laboratory in Krebs Henseleit bicarbonate buffer at 37 C which was continuously gassed with a mixture of 95 % O_2 and 5 % CO_2 The arteries were carefully freed from adventitia cut up longitudinally and divided into 4—5 mm broad pieces weighing 50—100 mg Adjacent pieces were used as test and control preparations Intact rat hemidiaphragm was used as described by Kono and Colowick (1961) The incubation procedure was the same as described earlier (Arnqvist 1971) briefly the tissue pieces were incubated in 25 ml flasks containing 4 ml Krebs Henseleit bicarbonate buffer The flasks were gassed with a mixture of 95 O_2 and 5 % CO_2 for 20 s sealed with tightly fitting rubber stoppers and then kept in an agitation bath at 37 C during the incubation period

Chemicals 3 0 methylglucose ^{14}C D glucose ^{14}C D glucose 1 ^{14}C L glucose 1 ^{14}C sorbitol ^{14}C and sorbitol ^{3}H were obtained from the radiochemical Centre Amersham England Instagel and Soluene were commercial preparations of Packard Instrument Company Inc Mono-component pork insulin (lot No Mc S 970 AC) was a gift from Novo Copenhagen

Tissue distribution of labelled substrates After the incubation in labelled substrate the tissue was weighed and dissolved in 1 ml Soluene 9 ml of scintillation fluid was added (toluene containing 5 g PPO and 0 3 g dimethyl POPOP per litre) The isotope content of the tissue was counted in a liquid scintillation spectrometer (Packard Tri Carb) 100 μl of the incubation medium was dissolved in 10 ml of Instagel and the amount of isotope was measured Duplicate samples were always counted The degree of quenching was determined by the external standard technique The counting efficiency was 65—75 The distribution of substrate in whole tissue (space) is expressed by the following equation

$$\textit{distribution} \ \% = \frac{\text{content in wet tissue (cpm/mg)}}{\text{medium concentration (cpm/}\mu\text{l)}} \cdot 100$$

The intracellular concentration of 3-0-methylglucose ^{14}C was calculated as follows

$$\text{Intracellular concentration} = \frac{MS - ES}{WW - ES} \cdot \text{medium concentration}$$ where WW is the total tissue water ES is the extracellular space and MS is the distribution of 3 0 methylglucose ^{14}C

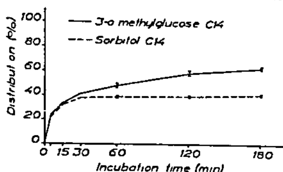


Fig 1 Distribution of 3 0-methyl glucose ^{14}C 10 mM and sorbitol ^{14}C 0 5 mM in bovine mesenteric arteries The sorbitol space was determined in the presence of 10 mM unlabelled 3 0-methyl glucose Mean ± S E (n = 5—7)

in whole tissue The intracellular concentration of 3-0 methylglucose was determined by a double isotope technique (Okita *et al* 1967) ^{14}C labelled 3 0 methylglucose and tritiated sorbitol were used The counting efficiencies were about 30 % for ^3H and 35 % for ^{14}C Total tissue water was determined by drying to constant weight at 100 C

Enzymatic determination of D glucose distribution After the incubation period the tissue was washed in buffer for 10 s and frozen at —80 C in frigen 12 containing solid CO_2 The frozen tissue was homogenized in 6 % perchloric acid After neutralization with K_2CO_3 the glucose content of the tissue extract was measured by the hexokinase glucose 6-phosphate dehydrogenase method (Slein 1962) Duplicate samples from the incubation medium were assayed for glucose by the same method The tissue distribution of glucose was calculated by the following formula

$$\text{distribution } (\%) = \frac{\text{content in wet tissue (mg/g)}}{\text{medium concentration (mg/ml)}} \quad 100$$

Chromatography To test whether 3 0-methylglucose ^{14}C was metabolized in rabbit colon smooth muscle and bovine mesenteric arteries tissue extracts were prepared and checked for metabolites by thin layer chromatography After an incubation period of 180 min in 10 mM 3 0 methylglucose ^{14}C the tissue was homogenized in 6 perchloric acid and neutralized with K_2CO_3 The proteinfree extract was spotted on precoated cellulose (Avicel) thin layer plates 20 by 20 cm and 250 μ thick (Analtech Inc) The plates were developed in butanol water glacial acetic acid 60 20 20 (Lewis and Smith 1967) The chromatograms were scanned with an isotope detector (Berthold thin layer scanner) Chromatograms of the tissue extracts showed radioactivity only in the spot corresponding to 3 0 methylglucose ^{14}C

Results

Distribution of 3 0 methylglucose in bovine mesenteric arteries The accumulation of ^{14}C labelled 3 0 methylglucose (10 mM) was studied at incubation periods varying between 5 and 180 min (Fig 1) Sorbitol ^{14}C (0 5 mM) was used to estimate the extracellular space in the presence of unlabelled 3 0 methylglucose (10 mM) The distribution of both 3 0 methylglucose ^{14}C and sorbitol ^{14}C increased rapidly during the first 30 min After that time the distribution of sorbitol ^{14}C reached an equilibrium and the sorbitol space remained practically constant for the rest of the incubation period After the first 30 min the distribution of 3 0-methylglucose exceeded that of sorbitol and continued to increase during the rest of the incubation time This indicated that 3 0 methylglucose but not sorbitol was able to penetrate the cell membranes in bovine mesenteric arteries The total tissue water was 78 1 ± 0 9 % (n = 6) after an incubation period of 180 min and the distribution of 3 0 methylglucose was 64 8 ± 0 9 (n = 10) As the concentration of 3 0

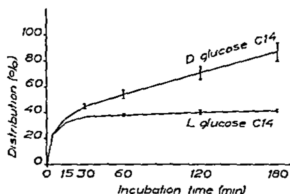


Fig 2 a Comparison between the accumulation of D glucose 1 ^{14}C and L glucose 1 ^{14}C in bovine mesenteric arteries The medium concentration of L or D glucose was 10 mM Mean ± S E (n = 6)

glucose in the extracellular space is supposed to be equal to the medium concentration 3 0 methylglucose was transported into the cells down a concentration gradient

Accumulation of D and L-glucose The accumulation of ^{14}C labelled D and L-glucose were studied in bovine mesenteric arteries and smooth muscle from rabbit colon The incubation time was varied between 5 and 180 min D glucose 1 ^{14}C and L glucose 1 ^{14}C were added to the incubation medium in a concentration of 10 mM After about 30 min the distribution of L glucose 1 ^{14}C in bovine mesenteric arteries (Fig 2 a) reached a constant level which corresponded to the sorbitol space (*cf* Fig 1) This indicates that L glucose is restricted to the extracellular space and does not penetrate the cell membrane to any significant degree The distribution of D glucose 1 ^{14}C exceeded that of L glucose 1 ^{14}C and continued to increase for at least 180 min The accumulation of D glucose 1 ^{14}C and L glucose 1 ^{14}C in smooth muscle from rabbit colon (Fig 2 b) closely resembled that in bovine mesenteric arteries These findings show that the membrane permeability in bovine mesenteric arteries and rabbit intestinal smooth muscle has substrate stereospecificity for monosaccharides

Counter transport It is possible to link the facilitated movement of a permeant down its concentration gradient with the movement in the opposite direction of a structually analogous molecule The second permeant is thus transported against its

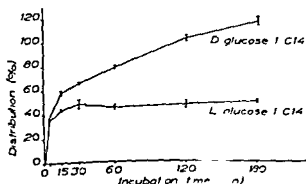
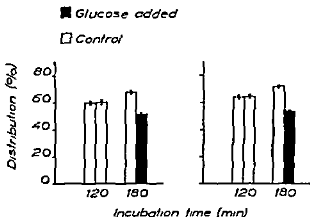


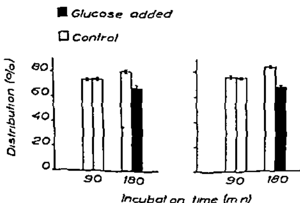
Fig 2 b Accumulation of D glucose 1 ^{14}C and L-glucose 1 ^{14}C 10 mM in smooth muscle from rabbit colon Mean ± S E (n = 6)

Fi 3 a Counter transport of 3-0-methylglucose ^{14}C (10 mM) in bovine mesenteric arteries on addition of glucose (20 mM) to the incubation medium After an incubation period of 120 min the distribution of 3-0-methylglucose ^{14}C was determined in a control pair To another pair glucose or saline was added after 120 min The incubation was continued and the distribution of 3-0-methylglucose ^{14}C was determined after 180 min The left diagram shows the results without insulin and the right diagram with insulin.



concentration gradient (Stein 1967) This phenomenon is called counter transport Counter transport was studied both in bovine mesenteric arteries (Fig 3 a) and in smooth muscle from rabbit colon (Fig 3 b) Four adjacent pieces from bovine mesenteric arteries were incubated in 3 0 methylglucose ^{14}C (10 mM) After 120 min the tissue distribution of 3 0 methylglucose was determined in one control pair 50 μl of a glucose solution was then added to one flask to make the final medium concentration of glucose 20 mM To the control preparation a similar amount of saline was added The incubation was continued for a further 60 min and the distribution of 3 0 methylglucose ^{14}C was determined in the last pair From Fig 3 a (left diagram) it is seen that after 120 min there was no difference in 3 0-methylglucose space in the control pair After 180 min the 3 0-methylglucose space had decreased significantly ($p < 0.01$) in the preparation to which glucose was added while it had increased in the control preparation This shows that 3-0 methyl glucose was transported out of the cells against a concentration gradient The same results were obtained in the presence of insulin (0 1 U/ml) (Fig 3 a right diagram) Counter transport was investigated in the same way in rabbit colon with the

Fig 3 b Counter transport of 3-0-methylglucose ^{14}C in smooth muscle from rabbit colon The experimental conditions were the same as in Fig 3 a, except that the distribution of 3 0-methyl glucose was determined after 90 min and that the glucose solution added at the same time gave a medium concentration of 10 mM Left diagram without insulin and right diagram with insulin Mean ± S E (n = 5)



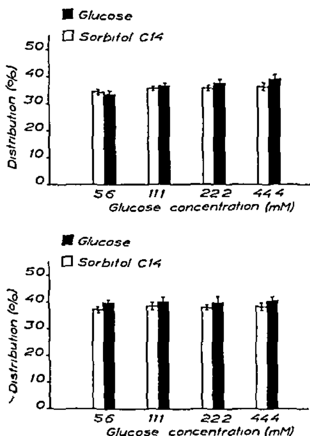


Fig 4 Comparison between the distribution of unlabelled glucose (5 6—44 4 mM) determined by an enzymatic method and the extracellular space estimated by sorbitol ^{14}C (0 5 mM) The spaces were compared after incubation times of 60 min (upper diagram) and 120 min (lower diagram) Mean±S E (n = 6)

exception that the 3 0 methylglucose space was determined after 90 min in the control pair and glucose was added after the same length of time Also in rabbit intestinal smooth muscle counter transport was demonstrated in the absence and presence of insulin (Fig 3 b)

Distribution of D glucose in bovine mesenteric arteries To study whether the membrane transport was rate limiting for the metabolism of glucose the tissue concentration of glucose was determined enzymatically by the hexokinase glucose 6 phosphate dehydrogenase method The glucose content of the tissue was calculated as glucose space and compared with the extracellular space The glucose space was studied in the concentration range 5 6—44 4 mM after incubation periods of 60 and 120 min The extracellular space was determined for each concentration by addition of 0 5 mM ^{14}C labelled sorbitol The results are shown in Fig 4 The glucose space tended to be somewhat higher than the sorbitol space but the difference was not significant If the phosphorylation of glucose by hexokinase was rate limiting for the glucose uptake glucose would accumulate intracellularly when the membrane transport exceeded the metabolic capacity The difference between glucose space and sorbitol space would then be expected to increase with time and increasing

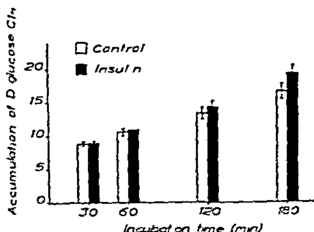


Fig 5 Effect of insulin (0 1 U/ml) on the accumulation of D-glucose-^{14}C in bovine mesenteric arteries The accumulation of ^{14}C-labeled glucose was calculated as 10^{-3} mg/100 mg wet tissue weight The effect of insulin was significant after 120 min ($p < 0\ 01$) and after 180 min ($p < 0\ 001$) Mean±S E (n = 6)

concentrations As no such increase was observed it seems probable that the membrane transport of glucose is rate limiting for the glucose metabolism in bovine mesenteric arteries.

Effect of insulin on D glucose ^{14}C accumulation in bovine mesenteric arteries In a previous study (Arnqvist 1971) insulin (0 1 U/ml) was found to stimulate D-glucose ^{14}C accumulation in rat aorta to a minor degree In smooth muscle from rabbit colon the effect of insulin was somewhat more pronounced It was therefore of interest to study the effect of insulin on glucose accumulation in bovine mesenteric arteries which have a high content of smooth muscle (Ducret 1930) The accumulation of D-glucose ^{14}C was studied at incubation times varying between 30 and 180 min (Fig 5) There was a significant effect of insulin after 120 ($p < 0\ 01$) and 180 min ($p < 0\ 001$ The increase in D-glucose ^{14}C accumulation due to insulin was 15 0±1 0 % after 180 min compared with 11 1±2.2 % in rat aorta and 22 7±4 0 % in smooth muscle from rabbit colon.

Effect of insulin on the distribution of 3-0 methylglucose ^{14}C in bovine mesenteric arteries To study to what degree the influence of insulin on the glucose accumulation could be attributed to an action on the monosaccharide transport the insulin effect on the distribution of 3-0-methylglucose ^{14}C (10 mM) was observed at incubation times varying between 30 and 180 min (Fig 6 a) Insulin (0 1 U/ml produced a small (2 7±0 6 but significant increase ($p < 0\ 01$) in the 3-0-methylglucose space after 180 min No effect of insulin was found on the extracellular space measured by sorbitol or on the total tissue water After 180 min without insulin the sorbitol space was 42 0±0 5 (n = 7) and the total tissue water 78 1 ± 0 9 (n = 6) With insulin the values were 43.2±0.9 (n = 7 and 77.9±0 7 (n = 6) This indicates that the increase in 3-0-methylglucose ^{14}C distribution was due to an effect of insulin on the membrane permeability

In skeletal muscle insulin has a large effect on the membrane permeability of monosaccharides (Kipnis and Cori 1957) To compare the effect of insulin on the

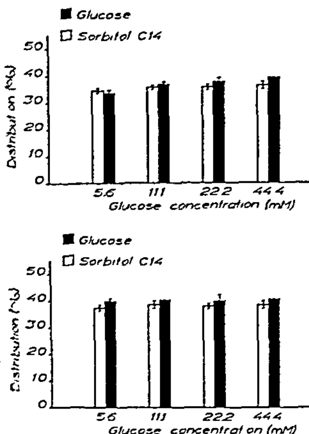


Fig 4 Comparison between the distribution of unlabelled glucose (5.6—44.4 mM) determined by an enzymatic method and the extracellular space estimated by sorbitol-¹⁴C (0.5 mM) The spaces were compared after incubation times of 60 min (upper diagram) and 120 min (lower diagram) Mean ± S E (n = 6)

exception that the 3-O-methylglucose space was determined after 90 min in the control pair and glucose was added after the same length of time Also in rabbit intestinal smooth muscle counter transport was demonstrated in the absence and presence of insulin Fig 3 b

Distribution of D glucose in bovine mesenteric arteries To study whether the membrane transport was rate limiting for the metabolism of glucose the tissue concentration of glucose was determined enzymatically by the hexokinase glucose-6-phosphate dehydrogenase method The glucose content of the tissue was calculated as glucose space and compared with the extracellular space The glucose space was studied in the concentration range 5.6—44.4 mM after incubation periods of 60 and 120 min The extracellular space was determined for each concentration by addition of 0.5 mM ^{14}C-labelled sorbitol The results are shown in Fig 4 The glucose space tended to be somewhat higher than the sorbitol space but the difference was not significant If the phosphorylation of glucose by hexokinase was rate limiting for the glucose uptake glucose would accumulate intracellularly when the membrane transport exceeded the metabolic capacity The difference between glucose space and sorbitol space would then be expected to increase with time and increasing

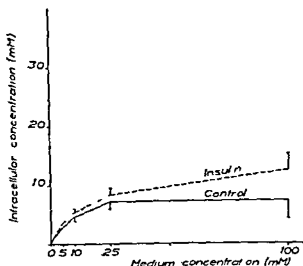


Fig 7 a. Intracellular concentration of 3-0-methylglucose ^{14}C in bovine mesenteric arteries after an incubation time of 180 min The concentration of 3 0-methylglucose was varied from 1 to 100 mM Insulin was added in a concentration of 0 1 U/ml. Mean ± SE (n = 6)

determined simultaneously with ^{3}H labelled sorbitol in a concentration of 0 5 mM The intracellular concentration of 3 0 methylglucose ^{14}C was measured after 180 min in the arteries and after 90 min in rabbit colon Fig 7 a and b show that with increasing medium concentrations the rise in intracellular 3-0-methylglucose-concentration became proportionately smaller indicating that the membrane permeability for monosaccharides was saturable Insulin in a concentration of 0 1 U/ml tended to increase the intracellular concentration of 3 0-methylglucose in both bovine mesenteric arteries and smooth muscle from rabbit colon At the highest concentration (100 mM) the effect of insulin was significant ($p < 0.05$) in rabbit colon smooth muscle (Fig 7 b)

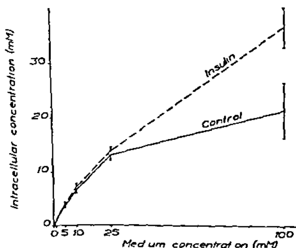


Fig 7 b Intracellular concentration of 3-0-methylglucose-^{14}C in smooth muscle from rabbit colon after an incubation time of 90 min The concentration of 3-0-methylglucose was varied from 1 to 100 mM Insulin was used in a concentration of 0 1 U/ml. Mean ± S E (n = 7)

Discussion

The results of this investigation show that the membrane permeability of monosaccharides in bovine mesenteric arteries and rabbit intestinal smooth muscle is characterized by substrate stereospecificity saturation kinetics and counter transport 3-0-methylglucose was transported down a concentration gradient in bovine mesenteric arteries In a previous work (Arnqvist 1971) it was found that the distribution of D xylose in rabbit intestinal smooth muscle rat and rabbit aorta did not exceed the water space D xylose therefore enter the cells of these tissues down a concentration gradient These findings show that the membrane permeability of monosaccharides in arterial tisue and smooth muscle has the characteristics of facilitated diffusion (Stein 1967)

The spaces of L-glucose and sorbitol were equal and constant for at least 150 min in both bovine mesenteric arteries and smooth muscle from rabbit colon This indicates that sorbitol and L-glucose did not penetrate the cell membranes It therefore seems probable that monosaccharides do not penetrate the cell membranes by simple diffusion to any significant degree

The space of unlabelled glucose was determined by an enzymatic method in bovine mesenteric arteries in an attempt to find out whether the membrane transport was rate limiting for glucose metabolism In the concentration range 5 6—44 4 mM the glucose space was of the same size as the extracellular space estimated by sorbitol ^{14}C This suggests that the membrane transport of glucose is rate limiting for the glucose metabolism

It was found earlier (Arnqvist 1971) that insulin increased the tissue distribution of D xylose in rabbit colon smooth muscle The effect was small but significant after incubation periods of 120 and 180 min Insulin also tended to increase the distribution of D xylose in rat aorta In this study insulin was found to stimulate the membrane transport of 3 0-methylglucose in bovine mesenteric arteries to a small but significant degree after an incubation time of 180 min It therefore seems probable that insulin in a high concentration (0 1 U/ml) can stimulate the membrane transport of monosaccharides in arterial tissue and intestinal smooth muscle In skeletal muscle insulin has a pronounced effect on the membrane transport of monosaccharides (Kipnis and Cori 1957) Insulin had only a small effect on the membrane tran port of monosaccharides in smooth muscle It was therefore of interest to compare the effect of insulin on a skeletal muscle preparation under the same conditions Intact rat hemidiaphragm was used for this purpose and in this tissue insulin had a strong effect on the membrane transport of 3-0 methylglucose after an incubation time of 30 min

The reason for the small effect of insulin on arterial tissue and smooth muscle *in vitro* might be that insulin only penetrated the tissue to a small degree In smooth muscle the size of the extracellular pace varies with the size of the molecules used as extracellular markers (Barr and Malvin 1965) Large molecules such as albumin give a low value for the extracellular space but the time taken for the extracellular space to reach a constant level does not differ between various sized mole

cules Furthermore if the small effect of insulin on smooth muscle and arterial tissue were due to poor penetration under *in vitro* conditions the effect of insulin would be expected to be stronger in a thin preparation than in a thick one The insulin effect was however even smaller in rat aorta than in bovine mesenteric arteries and this reason therefore does not seem to be valid

The monosaccharide permeabilities in smooth muscle from rabbit colon and bovine mesenteric arteries were found to have very similar characteristics and the effect of insulin in a high concentration (0 1 U/ml) was small in both tissues These results suggest that intestinal and arterial smooth muscle cell membranes are similar in this respect and that both are little affected by insulin The effect of insulin on D glucose ^{14}C accumulation was weaker in bovine mesenteric arteries than in rabbit colon smooth muscle This difference may be due to a lower smooth muscle content in arterial tissue than in the muscle layer from rabbit colon

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Acta physiol scand 1972 85 228—236

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The Pressure-Flow Relationship in Renal Cortical and Medullary Circulation

By

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Abstract

Grängsjo G and M Wolgast *The pressure flow relationship in renal cortical and medullary circulation* Acta physiol scand 1972 *85* 228—236

The perfusion pressure red cell flow relationship in the cortical and in the outer and inner medullary circulation has been investigated on 9 chloralos anesthetized mongrel dogs using an indicator-dilution technique with 32 P labelled red cells and internal detection with small needle-shaped semiconductor detectors The perfusion pressure was varied by a clamp on the aorta High pressures were obtained by ligation of the carotid arteries and section of the vagal preceded by denervation of the renal nerves The change in red cell flow on changing perfusion pressure was determined from 1 the change in the regional mean transit time and 2 the change in regional red cell volume The flow in all the regions studied was then found to be linearly related to the perfusion pressure up to some 70 mm Hg whereas in the pressure range between 0 and 200 mm Hg it remained essentially unchanged The findings indicate an autoregulative response in both the cortical and medullary circulation

Autoregulation of the total renal blood flow viz a relatively unchanged blood flow to the kidney while the perfusion pressure varies between about 80 and 200 mm Hg is a well established phenomenon (*e g* Thurau and Kramer 1959 a 1959 b Ochwadt 1956 Waugh 1964 Hinshaw 1964) This means that at least the cortical blood flow is autoregulated since about 90 % of the blood perfusing the kidney is bound for the cortical glomeruli and the cortical peritubular capillaries (Ullrich *et al* 1961 Lilienfield and Pommerantz 1963 Wolgast 1968) The pressure flow relationship of the medullary circulation however has been subjected to few investigations and the results are contradictory

Using a dye dilution technique with detection of the dye on the pelvic surface of the medulla Thurau Deetjen and Kramer (1960) found a relatively linear relationship between the perfusion pressure and the medullary blood flow expressed as the reciprocal of the mean transit time recorded This finding could explain the linear relationship between perfusion pressure and urine flow (Thurau and Deetjen 1962) Another interesting theory which predicts the same finding is that the vascular resistance is determined by the sodium concentration at the macula densa

segment of the nephron via the release of renin from the juxta glomerular cells Since the renin concentration in the glomeruli of the medullary vascular bed (i e the juxtamedullar glomeruli) is low (Bing and Wiberg 1958 Peart 1959), it seems likely that the glomerular filtration rate in this region and the medullary blood flow are not autoregulated In contradiction of this view an equally well developed autoregulation in the cortical and medullary circulation has been found by Aukland (1966) and more recently by Løyning (1971) on measuring the local clearance of hydrogen from different areas of the kidney and by Wolgast (1968) using an indicator dilution technique with labelled red cells and detection with small needle shaped beta sensitive detectors

The preceding investigations dealt primarily with the pressure flow relationship at pressures below 150 mm Hg The purpose of the present investigation however was to study this relationship in the cortical and in the outer and inner medullary circulation within a larger pressure range from 20 to 200 mm Hg

Material and Methods

The expts were performed on 9 dogs—harrier and schaler weighing 21±6 kg (mean±S D) and fed on a standard diet. Anesthesia was induced by thiopental sodium (Pentothal sodium® Abbott laboratories Ltd Great Britain) in a dose of 10—15 mg/kg and maintained with 1 % chloralose (E Merck AG West Germany) in saline The chloralose was given in an initial dose of about 50 mg/kg and was followed by smaller maintenance doses A tracheal tube was inserted to ensure free airways Catheters were then inserted in (1) the cubital vein for infusion of solutions (2) the femoral artery for recording the perfusion pressure in the renal artery and (3) both ureters for urine collection

The left kidney was exposed by a flank incision A fine bore catheter (external diameter 0 75 mm) was inserted into the renal artery via a lumbar artery for the injection of the P^{32} labelled red cells Three beta sensitive semiconductor detectors (either 3 separate 1 5 mm thick detectors or a triple detector containing 3 separate elements) were inserted *one* into the middle of the cortex *one* into the outer medulla and *one* into the outer layers of the inner medulla The detectors were usually inserted parallel to the striated direction of the kidney with their tips oriented towards the tip of the papilla. The monitored volume using P^{32} as the radioactive source (maximum beta energy 1 7 MeV) corresponds to an oblate spheroid having a 7—8 mm major axis perpendicular to the needle detector s long axis and a 5—6 mm minor axis The detected region is considered to be representative for a 5—6 mm thick layer cut perpendicular to the striatal direction of the renal parenchyma (Wolgast 1968)

Red cells were labelled by mixing 2 ml of the cells with 0 2—0 5 ml of a citrate phosphate buffer solution (Mollison *et al* 1958) to which approximately 15 mC P^{32} phosphate had been added After incubation at 38 C for 1 5 h the red cells contained at least 75 % of the radioactivity The labelled red cells were then washed three times in cold saline and were resuspended in 2 ml of their own plasma.

In order to obtain an indicator-dilution curve of sufficient accuracy* (which means a total of about 3000 counts) 0 3—0 5 ml of the radioactive blood had to be injected at each determination

The pulses from the detectors were recorded by a three-channel analyzing apparatus from AB Atomenergi Studsvik Sweden consisting of charge sensitive preamplifiers (Mod 4650 C) main amplifiers (Mod 4631 B) discriminators (Mod 4106 A) and rat meters (Mod 4603 D)

* The amount of radioactivity M needed for obtaining a certain number of counts A can be calculated by the equation

$$A = \frac{M \cdot V_{rr} \cdot E}{F}$$

where V_{rr} is the fraction of red cell volume in the organ E the mean efficiency of the detector and F the red cell flow where the indicator is to be injected (Wolgast 1968)

The signals from he ratemeters were fed to a photokymograph (Ultralette Mod 5650 ABEM Solna Sweden)

The perfusion pressure was varied by a clamp on the aorta placed proximal to the departure of the artery High perfusion pressures were obtained by ligation of the carotid arteries proximal to the carotid sinuses and cutting of the vagal nerves preceded by surgical and pharmacological denervation of the renal nerves—the latter by infiltration of approximately 2 ml of a 4 % lidocaine solution (Xylocain® AB Astra Sodertalje Sweden

Experimental Procedure

After completion of surgery and insertion of the experimental device the kidney was allowed to return to its natural position and the wound was temporarily closed Usually 1—3 regional mean transit time determinations at the original systemic blood pressure were made by rapid injection of labelled blood cells into the renal artery s fine bore catheter The indicator dilution curves from the detectors were recorded for 2—4 min The determinations were then repeated either at pressures below the systemic blood pressure or as in most of the expts at the highest pressure obtained (up to about 200 mm Hg) Subsequent determinations were made alternately at low and high pressures

The change in regional blood volume at the different pressures was determined as the change in the recorded background activity from the detectors when the labelled cells had been equilibrated throughout the total blood volume of the body

Calculations

In order to obtain indicator dilution curves for a single circulation the curves were extrapolated to zero assuming a monoexponential decrease No compartment analysis was made since the aim of these studies was to determine the relationship existing between curves rather than to make an exact determination of the mean transit time in different compartments within the monitored volume

The mean transit time was calculated in several ways, among which the value obtained by dividing the area of the curve by the peak activity ($t_{A/H}$) was employed in subsequent calculations The regional blood flow has been expressed as the reciprocal of the mean transit time where the mean value was taken as 100 % when the perfusion pressure equaled the original blood pressure Values obtained at pressures higher or lower than this pressure were calculated from (1) the change in the reciprocal of the new mean transit time and (2) the change in background activity Indicator-dilution curves have been excluded that were generated to a large part by radioactivity passing through large vessels (which could especially be obtained in recordings from detector positions deep in the inner zone) as well as curves consisting of a small number of counts

Results

The mean values for the mean transit time calculated as $t_{A/H}$ as a function of the distance from the cortico medullary border at perfusion pressures equal to the original systemic pressure are given in Fig 1 In the cortical parenchyma the mean transit time is rather short—the mean value in these series being 3 0 s In the medulla the mean value increased from 7 1 s in the middle of the outer zone (3 mm from the cortico-medullary border) to 20 7 s in the outer parts of the inner zone (9 mm from the cortico-medullary border)

Using the values for regional red cell volume given by Emery *et al* (1959) (i e 8 0 % 6 0 % and 6 0 % for the cortex outer medulla and inner medulla respectively) and assuming the hematocrit of the blood perfusing all these regions to be 0 40 the corresponding blood flows were calculated to be 4 1 1 3 and 0 4 ml/min gram tissue

Fig 2 shows the indicator dilution curves recorded from the cortex (I) outer medulla (II) and inner medulla (III) at perfusion pressures of 190 mm Hg and 135 mm Hg (The perfusion pressure was reduced to 135 mm Hg by a clamp on the

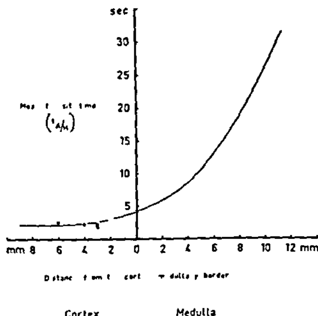


Fig 1 Mean values of the mean transit time obtained at control conditions as a function of the distance from the cortico-medullary border

aorta.) The cortical mean transit time increased from 2 6 to 2 8 s The outer medullary transit time showed an increase of the same order of magnitude viz from 5 0 to 5 3 s but in the inner zone the mean value changed from 31 3 to 37 1 s—thus a comparatively larger increase

In the experiment in Fig 2 the red cell volume did not change significantly therefore the reciprocal of the mean transit time values can be taken as an index of the change in the regional blood flow This means that when the perfusion pressure was reduced from 190 to 135 mm Hg (corresponding to a 29 % decrease in perfusion pressure) the cortical blood flow was reduced by 7 3 % the blood flow in the outer medulla by 5 6 % and that in the inner medulla by 15 6 % The blood flow in all these areas was thus not linearly related to the perfusion pressure but exhibit the autoregulative pattern of pressure flow relationship

The pressure flow relationship obtained from all the experiments have been plotted in Fig 3 Values obtained when the perfusion pressure equaled the original systemic pressure are represented by the unfilled circles

The values given were primarily determined by changes in the mean transit time since the red cell volume showed relatively minor alterations (for the most part less than 5—10 %) Within the autoregulation range the red cell volume usually remained constant or increased slightly at lower pressures a moderate decrease or increase was recorded The values show a rather large scatter due to the fact that all experiments have been plotted together in the same curve and perhaps to other reasons as the redistribution of blood flow within the different areas of the kidney etc This does not inhibit the conclusion being drawn that the blood flow in all

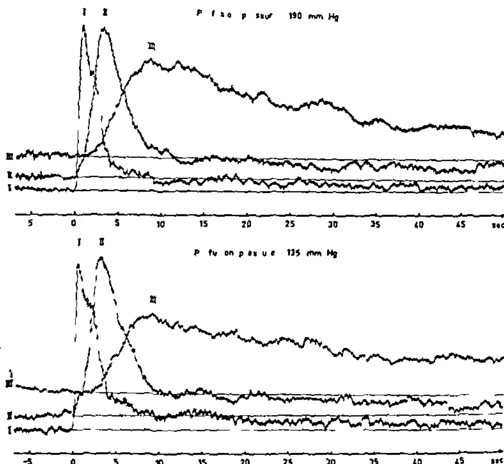


Fig 2 Cortical (I) outer medullary (II) and inner medullary (III) indicator dilution curves obtained after a slug injection of P^{32} labelled red cells at time zero In the upper panel the curves at 190 mm Hg are visualized and in the lower the corresponding curves after reducing the perfusion pressure to 135 mm Hg

of the kidney increases relatively linearly with perfusion pressure up to about 70 mm Hg Within the 70—200 mm Hg pressure range the blood flow is relatively constant

Fig 4 shows the relative cortical blood flows at different perfusion pressures compared with the corresponding medullary blood flow values The autoregulation of the blood flow seems to be as equally well developed in the medullary circulation as in the cortical circulation

Discussion

As previously pointed out very few investigations have been made on the relationship between perfusion pressure and blood flow in different regions of the kidney This is in contrast to the immense amount of literature on the pressure flow rela

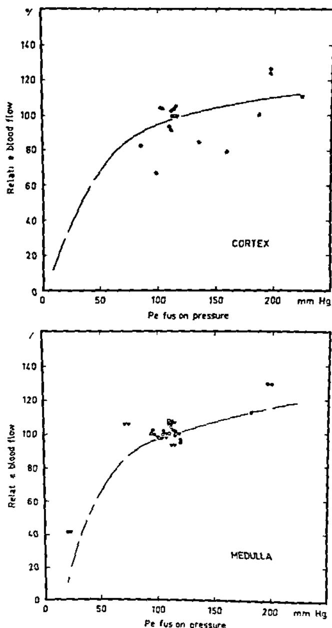


Fig 3 Pressure flow relationship in the cortical (upper panel) and medullary (lower panel) circulation Unfilled symbols denotes the values obtained at control pressures and are taken as 100% on the average In the latter figure (lower panel) the circles denote values from the outer medulla and triangles from the inner medulla.

tionship as it relates to the total renal circulation The apparent discrepancy can certainly be attributed to the technical and analytical difficulties in regional blood flow determinations Methods based on inert diffusible indicators as used by Aukland (1964 hydrogen clearance technique) and Grangsjo (1968 heated thermocouples) have the advantage that the volume of distribution of the indicator can be

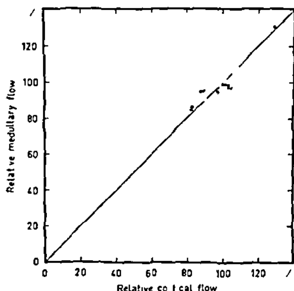


Fig 4 Relationship between cortical and medullary flow at the different *perfusion pressures induced Circles* denote the cortico-outer medullary and the triangles the cortico-inner medullary relationship

equalized with the tissue volume so that the blood flow can be calculated directly the curves obtained The thermocouples have, further the advantage of continuous recording but otherwise they do not differ in principle from techniques involving the measurement of local clearance of a diffusible indicator In the kidney, however the urine flow influences the flow determined—at least as far the inner medulla is concerned (Aukland 1964)

The well known linear relationship between urine flow and perfusion pressure then masks and possible autoregulation of the inner medullary blood flow when using methods based on inert diffusible indicators Another source of error inherent in these methods is the influence of the damaged zone around the detectors in the parenchyma In a large damaged zone the calculated blood flow probably represents an underestimation of the true blood flow As the curve obtained is partly dependent on the diffusion characteristics of the indicator an increase in the true blood flow *would also be underestimated Upon comparison between the values obtained in the* outer medulla by the hydrogen clearance technique and those by the indicator dilution technique outlined in this paper the relative changes in blood flow in the two methods did not however differ essentially (Aukland and Wolgast 1968)

When using intravascular indicators (as in the present technique and in the photoelectric technique employed by Thurau Deetjen and Kramer (1960) the indicator cannot be distributed in the damaged zone where the blood flow is stopped As in the photoelectric technique parts of free radioactivity or dye may escape however from the vascular compartment and cause errors in the blood flow determinations It should be pointed out that these methods involve both the determination of the mean transit time of the indicator through the monitored volume and the volume of distribution of the indicator

In the present investigation changes in the regional blood volume were small and therefore the relative blood flow may well be expressed as the reciprocal of mean transit time — as was done by Thurau Deetjen and Kramer (1960) The results from these two investigations seem therefore to be comparable since the experimental conditions were essentially the same The discrepancy between the obtained results remains as yet unexplained The fact that in the present investigation the indicator consisted of labelled red cells whereas the photoelectric technique uses a protein bound dye should probably make no difference

The results of these experiments do not permit any important conclusions about the mechanism of renal autoregulation They possibly contradict one consequence of the interesting theory for renal autoregulation outlined by Thurau (Thurau and Schnermann 1965 Thurau *et al* 1967) That theory postulates that the sodium concentration (or load) at the macula densa segment of the nephron should determine the release of renin from the juxtamedullary apparatus thereby determining the preglomerular vascular resistance As the concentration of renin is low in the juxtaglomerular apparatus of the juxtamedullary glomeruli there should be no well developed autoregulation of the blood flow to the medulla which is consequently in disagreement with the findings in this study

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Acta physiol scand 1972 85 237—241

From the Nobel Institute for Neurophysiology Karolinska Institutet Stockholm Sweden

The Effect of Calcium on the Potassium Permeability in the Myelinated Nerve Fibre of Xenopus Laevis

By

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Received 18 November 1971

Abstract

BRISMAR T and B FRANKENHAEUSER *The effect of calcium on the potassium permeability in the myelinated nerve fibre of Xenopus laevis* Acta physiol scand 1972 *85* 237—241

The effect of changes in the external calcium concentration (in the range from 0 27 to 109 mM) on the potassium permeability mechanism has been investigated with potential clamp technique An increment in [Ca] was associated with a positive shift along the potential axis of the curve relating the potassium permeability variable (n_∞) to the membrane potential This shift varied between 3 mV per e fold change in [Ca] at low concentrations and 10 mV in the range of high [Ca] Changes in magnesium concentration were associated with a similar shift

Most effects of calcium on the ionic currents in the squid giant nerve fibre are described by the following statement a change in the external calcium concentration is associated with a shift along the potential axis of the curves by which sodium conductance and potassium conductance are related to membrane potential (Frankenhaeuser and Hodgkin 1957) This statement is based on an analysis of the ionic currents during step changes of the membrane potential Indirect evidence indicates that calcium has a similar effect on the node of Ranvier in the myelinated nerve fibre This evidence was obtained from measurements of nodal excitability rectification action current and action potential with the node in solutions with various calcium concentrations (Frankenhaeuser 1957 a b Ulbricht 1964) However Hille (1968) found in potential clamp experiments that the potassium current is unaffected by the calcium concentration while the sodium current shows the predicted changes This absence of a calcium effect on the potassium current seems to be contradictory to the earlier finding that calcium does affect the rectification observed with slowly increasing currents (Frankenhaeuser 1957 b)

Potential clamp experiments were carried out in order to reanalyse the effect of calcium on the potassium currents in the myelinated nerve fibre

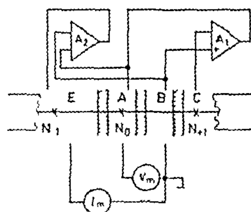


Fig 1 Circuit diagram of voltage clamp system The single nerve fibre mounted in perspex chamber with four pools (E A B C) Node under investigations N_0 N_{-1} and N_{+1} adjacent nodes Feed back amplifiers A_1 and A_2 Recording amplifiers V_m and I_m

Methods

The *membrane potential of single nodes of Ranvier in large isolated nerve fibres* was changed in rectangular pulse steps The technique for doing this was essentially the same feed back technique as described by Dodge and Frankenhaeuser (1958) i e a measurement of membrane potential was obtained with one feed back system and potential control was achieved with a second amplifier Operational amplifiers were used instead of valve amplifiers Amplifier drift and instrumental noise were decreased by this The decreased amplifier drift allowed a different procedure to balance the feed back amplifiers compared to that previously used

Figure 1 is a simplified circuit diagram of the system used Amplifiers A_1 and A_2 are the feed back amplifiers required for the potential clamp and amplifiers V_m and I_m are used for monitoring membrane potential and membrane current Calomel half cells and KCl starch gel bridges were used between the solution pools (E A B and C) and amplifier inputs and outputs Current carrying (output) electrodes were separate from measuring (input) electrodes (not shown in Fig 1) Follower amplifiers were used between electrodes and feed back amplifier inputs

The procedure to balance the amplifiers was

(1) Input electrodes B A and E were connected by KCl bridges to output electrode B Amplifiers V_m and I_m were balanced to a suitable point indicating zero potential at the input

(2) *Outputs A B and E were connected to inputs A B and E respectively Output F* was connected to C Both amplifiers were balanced to zero

The amplifiers were balanced prior to the experiments As a rule only small readjustments were necessary

The nerve fibre was cut off in pool C in order to decrease the resistance between a point (D) in the axis cylinder at the node under investigation (N_0) and the electrode C The fibre was further cut off at node N_{-1} in order to obtain a constant resistance between pool E and the point D A standard value of the product nodal surface area (A_N) times impedance between E and D (Z_{ED}) of 14 Ω cm² could thus be used This value is required for current calibration (Dodge and Frankenhaeuser 1959 Frankenhaeuser 1962) Isotonic KCl solution was used in pools C and E and in pool B to avoid liquid junction potential over seal BC

Solutions The Ringer's solution used had the following composition (mM) NaCl 112 $NaHCO_3$ 2 5 KCl 2 5 $CaCl_2$ 2 0

The clamp runs were taken with the node N_0 in solution with low sodium concentration and high potassium concentration (2 5 mM $NaHCO_3$ and 114 5 mM KCl) A number of such solutions with different calcium concentrations were used The calcium concentration was varied in these solutions in e fold steps between 0 27 and 109 mM (0 27 0 74 2 0 5 4 14 8 40 1 109 mM Note that the solutions with high [Ca] had a higher osmolarity than the other solutions Corresponding solutions with magnesium instead of calcium were used in *some experiments*

Nomenclature Potentials are given as inside potential minus outside potential Potentials relative to resting membrane potential are denoted V and in absolute values E thus V = E $-E_r$ Outward currents are consequently given as positive

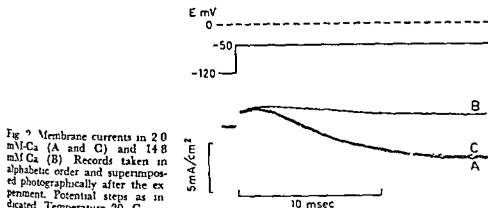


FIG 2 Membrane currents in 2 0 mM-Ca (A and C) and 14 8 mM Ca (B) Records taken in alphabetic order and superimposed photographically after the experiment. Potential steps as indicated Temperature 20 C

Results

The aim of the experiments to be described is to analyse the effects of changes in the external calcium concentration on the potassium permeability mechanism. Solutions with high potassium concentration (114 5 mM) and low sodium concentration (2 5 mM) were applied to the clamped node (pool A in Fig 1) Such solutions were preferred compared to the ordinary Ringer s solution because the sodium currents are so small that they almost can be neglected and the electrochemical driving force for potassium is appreciable at the potential region where potassium permeability changes much with the potential It is therefore easier to obtain reliable measurements with these solutions than with solutions with high [Na] and low [K]

Figure 2 shows three after the experiment photographically superimposed records of membrane current associated with step changes of membrane potential from E = −120 mV to E = −50 mV The records were taken in the order A B C High [K] (114 5 mM) and low [Na] (2 5 mM) were used throughout The [Ca] was 2 0 mM in A and C while it was 14 8 mM in B It is clearly seen that the potential step was associated with an appreciable inward current in the records taken with the low [Ca] while record B high [Ca] shows only a small potassium current The potential step to —50 mV was chosen for the Figure because it distinctly shows a region where the turn on of the potassium mechanism depends on the external [Ca]

Measurements were also made with the fibre in solutions with 114 5 mM Na and 2 5 mM K at depolarizations to the sodium equilibrium potential The n_{∞} curve obtained with conditioning pulses of different size showed a similar [Ca] dependent potential shift

The finding described in Fig 2 indicates that changes in [Ca] affected the potassium currents in the nodal membrane A more complete analysis of the calcium effect was therefore carried out The dimensionless variable n_{∞} was determined as previously described (Frankenhaeuser 1963) with the fibre in a number of different calcium concentrations (Fig 3) It is seen that the effect of calcium may be described by a shift along the potential axis of the n_{∞} -curve This shift caused by changes in calcium concentration was measured on four axons and is plotted in Fig 4 The

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From the Department of Pharmacology University of Lund Sweden

A Note on the Influence of Asynchronous Activation on Myocardial Contraction

By

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Abstract

JOHANNSSON M and E NILSSON *A note on the influence of asynchronous activation on myocardial contraction* Acta physiol scand 1972 85 242—248

Pairs of rabbit papillary muscles were mounted in series to make it possible to study the effects of asynchronous activation on myocardial contraction The two muscles were connected together *via* platinum loops hooked to a lever (displacement transducer) the other end of each muscle being fixed to a force transducer The force of each muscle was measured at various contraction frequencies with the lever fixed (isometric contractions) or freely movable (auxotonic contractions) With auxotonic contractions asynchronous activation (25 or 50 ms interval between stimulation of the two muscles) increased the movements of the muscles decreased their force and prolonged the duration of the contraction at 37 C These effects were greater the higher the contraction frequency within the range 30—150 beats/min On the basis of these results it is suggested that the physiological asynchrony of the myocardial contraction *in vivo* tends to offset frequency induced changes at the cellular lever (enhanced intensity of active state shortening of active state duration)

Wiggers (1927 a) considered tension development in the wall of the mammalian ventricle as the sum of the contractile responses of many small muscle sections sequentially activated during excitation of the ventricle As judged from extracellular recordings of the ventricular surface the excitation of a mammalian ventricle is completed within approximately 50 ms (Scher 1962) As a result of the sequential activation of the ventricle one would expect the parts of the ventricle that are first activated to stretch other areas during development of intraventricular pressure Rushmer (1956) also showed that during the isovolumetric contraction phase of a ventricle there are great changes in its dimensions —Little information is available however concerning the influence of asynchronous activation on myocardial contraction In the present study the effects of asynchrony is analysed *in vitro* A preparation is used consisting of two series-coupled papillary muscles which can be stimulated with different time intervals It will be demonstrated that at 37° C the amplitude of the contraction of these muscles is reduced and its time course prolonged when the muscles are stimulated 25 and 50 ms apart Furthermore these effects of asynchrony tend to increase with increasing frequency within the range 30—150 contractions/min

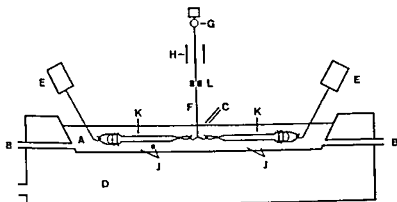


Fig 1 Schematic illustration of muscle chamber and recording arrangements used in the experiments A Muscle bath B Inlets for Ringer's solution C Suction drain. D Thermostatically controlled waterjacket E Force transducers (RCA 5734) whose positions were controlled by microscrews F Lever G Fulcrum of lever H Capacitor plates for recording the position of the (earth connected) lever J Stimulation electrodes K Papillary muscles L Releasable stop fitting on both sides of the lever

Methods

Isolated papillary muscles of rabbits were used The rabbits (weight less than 1 2 kg) were heparinized before sacrifice and the heart was immediately removed and opened in oxygenated Ringer's solution Papillary muscles from the right ventricle were dissected out together with a small portion of the ventricle wall near the base of the muscle and the tendinous extension at the tip of the muscle Platinum loops were firmly tied with silk thread to the tendon and to the piece of the ventricular wall as closely as possible to the insertions of the muscle The resting length of the muscles used as determined with a microscope (magnification 10 ×) with the muscles stretched by a preload of 100 dyn varied within the range 3 ?—6 7 mm the diameter of the preparation was 0 4—1 2 mm Usually only one suitable papillary muscle could be obtained from each heart

In the beginning of this study a few trabecular muscles from rabbit hearts were also dissected essentially as described above The relation between contraction frequency and isometric force was found to be very similar in trabecular and in papillary muscles therefore only the latter which can be more easily obtained without injury to the preparation were used in the actual experiments

For each exp two papillary muscles were mounted horizontally in a thermostated lucite bath see Fig 1 The platinum loop at the tendon end of a preparation was hooked to the tip of a lever and the loop at the ventricular wall end was connected to the extended peg of a mechano electronic force transducer (RCA 5734) The length of the preparation was adjusted by moving the force transducer along the length axis of the bath

The compliance of the two force transducers was 5 and 10 $\mu/10^3$ dyn The static friction of the lever was 3 5 mg The resonant frequency of the lever was not determined but exceeded 25 Hz with Ringer's solution in the recording bath and no preparation connected to the lever Movements of the lever were recorded by means of a capacitance position transducer

The signals from the 2 force transducers and the length transducer were recorded together with stimulation signals on an oscillograph (Mingograf® flat frequency response up to 500 Hz)

Each papillary muscle was stimulated by passing current through a pair of platinum wire electrodes situated beneath the midpoint of the muscle Rectangular pulses of ms duration were used voltage strength was 50 % above threshold value The time interval between stimulation of the two muscles as well as the order of stimulation of the two muscles could be varied

The muscles were stimulated at a rate of 60 beats/min for at least 1 h after being mounted in the recording device before the exp was started. The effects to be described refer to steady state conditions at the contraction frequency used

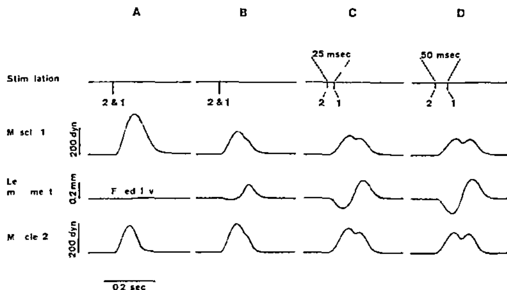


Fig 2 Force time curves of two series-coupled papillary muscles demonstrating influence of stimulation interval Upward deflection of third record indicates shortening of muscle 1 Contraction frequency 100 beats/min resting force in all recordings 50 dyn resting length of 1 and 2 5 9 and 6 0 mm respectively Note different force scales for muscle 1 and 2 Isometric contractions with synchronous stimulation (lever fixed) B—D Lever freely able

A Ringer s solution of the following composition was used (mM) NaCl 120 KCl 4 $NaHCO_3$ 20 NaH_2PO_4 1 5 $MgSO_4$ 1 5 $CaCl_2$ 2 glucose 3 3 The solution was continuously aerated with a mixture of 95 % O and 5 % CO_2 during dissection of the muscle and during the actual exp in order to keep the pH of the solution at 7 4—7 5 During the expts Ringer s solution flowed through the bath at a rate of 1—3 ml/min The temperature of the solution in the bath was continuously monitored and kept constant at 37±0 3 C Glass distilled water was used for washing the glassware and for preparing the solutions All chemicals were of analytical grade

Results

Records from one typical exp are shown in Fig 2 Panel A shows isometric contraction of the two muscles (lever fixed) When the muscles were stimulated simultaneously with the lever freely movable (panel B) the maximum force of the preparation was intermediate between the peak isometric forces of the two muscles With increasing time interval between the stimulation of the two muscles (panels C and D), the total movement of the lever i e the sum of its deflections in both directions increased and the maximum force of the preparation decreased Both the maximum force of the preparation and the rate of force development were reduced by increasing the time interval between stimulation of the muscles wheras the total duration of the contraction was prolonged by this intervention Six expts of this type were performed and in all the force of the preparation diminished with increasing lever movements during the contraction irrespective of order of stimulation of the muscles

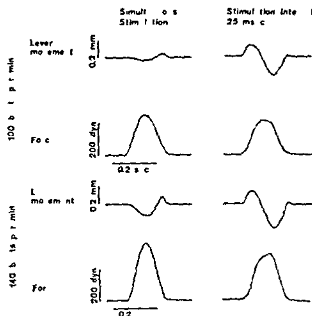


Fig 3 A Force time curves of two series-coupled papillary muscles illustraing the effects of asynchronous activation at frequencies 100 and 140 contractions/min Only records from one force transducer are shown Note the greater force reduction at the higher contraction frequency Resting force of the two muscles 100 dyn, resting length 3 2 and 4 2 mm respectively

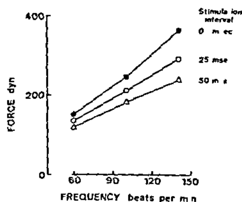


Fig 3 B Influence of stimulation asynchrony on maximum active force of two series coupled papillary muscles at contraction frequencies 60—140 beats/min Same exp as in Fig 3 A

The effects of asynchronous activation were observed at high resting forces as well as at low In one typical exp in which each of the two muscles were stretched to lengths near the optimum for active force stimulation intervals of 25 and 50 ms reduced maximum active force by 12 and 20 % respectively Contraction rate during this exp was 100 beats/min

The effects of asynchronous activation became more and more pronounced with increasing contraction frequency Fig 3 A shows traces from 1 exp in which the interval between stimulation of the 2 muscles was varied at different frequencies a in Fig 3 B the maximum force of the muscles is plotted as function of frequen

Discussion

The preparation used in the present study (2 papillary muscles arranged in series) was used in order to simulate a segment of the myocardial wall whose parts might be excited at different times As the peak isometric force of trabecular strips changed with frequency in a manner very similar to that of papillary muscles it can be assumed that the results of the present study are applicable to ventricular myocardium in general The results demonstrate that with asynchronous activation there is an increased movement of the 2 papillary muscles and a diminution of the maximum force produced This can be explained by presuming that with the experimental conditions used (temp 37° C contraction frequency 30—150 beats/min) the time course of the active state in each contractile element will be clearly separated in time when the muscles are excited 25 or 50 ms apart Consequently at any instant the muscle with the greatest force producing capability will succeed in stretching the other muscle

The finding of the present study that an interval of 25 or 50 ms between the excitation of two series coupled papillary muscles decreases the force of the preparation is in apparent contrast to results of a previous investigation (Tyberg Parmley and Sonnenblick 1969) These authors concluded that physiological degrees of asynchrony (stimulation intervals up to 60 ms were used in their study) did not reduce the force developed by a preparation consisting of 2 papillary muscles arranged in series It should be pointed out however that these results were obtained at a thempratture of 29 C and a contraction frequency of 12 beats/min and during such conditions the duration of contraction in myocardial preparations is very long Therefore the ratio between stimulation interval and duration of the contraction of the papillary muscles (measured at e g 50 % of maximum intensity) was much greater in the present expts than in those of Tyberg Parmley and Sonnenblick (1969) even though the stimulation intervals used were approximately equal

A complete mechanical analysis of the contraction of the two series coupled muscles is beyond the scope of this report Such an analysis would have to take into account the following factors 1) Shortening or stretching of a muscle reduces or enhances its mechanical performance by bringing the contractile element to shorter or longer lengths respectively (see Nilsson 1972) 2) Active shortening reduces the degree of activation of the contractile element (Brady 1966 Edman and Nilsson 1971) 3 Deactivation is also induced by passive stretch if the displacement occurs late in the contraction cycle (Brady 1966) The maximum force of the first stimulated muscle will be reduced with respect to isometric conditions because its maximum active state intensity is reached at a shorter length than during isometry and because of active shortening and passive stretch during the later part of its contraction cycle The other muscle will be excited at a longer and mechanically more advantageous length than during isometry but its force producing capability is reduced by shortening during the auxotonic contraction

The effects of a given stimulation interval (length changes of the two muscles and depression of their force during auxotonic contraction) were found to be greater the

higher the contraction frequency This can be because the duration of the mechanical activity of each of the papillary muscles decreases and the intensity increases with frequency Hence the contractile asynchrony is augmented at high rates —In the whole heart intraventricular conduction velocity is unaffected by frequency changes or slightly reduced at high rates (Wiggers 1927 b Hoffman and Suckling 1954 Gennser and Nilsson 1970 Rosen *et al* 1970) Therefore contractile asynchrony increases with frequency also in the intact heart The asynchrony of the ventricular contraction may be considered to moderate frequency induced cellular effects (enhanced intensity of active state shortening of active state duration) The asynchrony tends to prolong the ventricular systole thereby opposing the influence of the reduced duration of the active state in heart cell Conversely the increased intensity of the active state at high frequencies counteracts the negative effect of asynchrony on the rate of pressure development. In this connection it is of intrest that in a recent study on dog hearts the frequency dependence of isometric force of trabecular muscles was found to differ from that of intraventricular pressure during isovolumetric contractions (Kavaler *et al* 1971) Increments of contraction frequency that markedly enhanced peak isometric force of the trabecular muscles did not affect the maximum intraventricular pressure

In a number of recent investigations the mechanical properties of contracting ventricles have been analysed in terms of muscle models derived from work on isolated skeletal muscle and myocardial preparations (for references see Braunwald Ross and Sonnenblick 1968 Sonnenblick Parmley and Urschel 1969) These investigations of the mechanics of ventricular contraction are all implicitly based on the assumption that a ventricle is activated synchronously and symmetrically (*cf* p 496 Sonnenblick Parmley and Urschel 1969) Conversely the present study indicates that asynchronous activation is of major importance for ventricular wall stress and hence for intraventricular pressure during myocardial contraction Consequently intraventricular pressure might be altered by any factor that changes the degree of asynchrony of the myocardial contraction It has been suggested that increased synchronicity could significantly contribute to the enhanced ventricular contractility observed during sympathetic activation due to stimulation of stellate ganglia (Randall and Priola 1965)

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Acta physiol scand 1972 85 249—257

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Thermoregulation during Positive and Negative Work at Different Environmental Temperatures

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Abstract

NIELSEN B S L NIELSEN and F B PETERSEN *Thermoregulation during positive and negative work at different environmental temperatures* Acta physiol scand 1972 85 249—257

Cardiac output and thermoregulatory reactions were studied at 20 30 and 35 C during positive work (pos I) and negative work (neg) at O_2 uptakes of 0 8 l/min and also during positive work (pos II) at an O uptake of 1 7 l/min) i e with a total heat production equal to that during the negative work. At equal O uptakes the cardiac output ($\dot{Q}$) was the same in pos I and neg The blood flow in the working muscles estimated with a Xe method was not measurably different in pos I and neg The upper limit for thermal equilibrium was reached at lower environmental temperature in negative work This is most probably explained by the higher skin circulation during negative work Since the total $\dot{Q}$ and muscle blood flow is the same in pos I and neg the blood reserve available for heat transport during external heat stress e g in splanchnic organs is presumably reduced in negative work

In positive work e g walking uphill or pedalling a bicycle ergometer the amount of energy liberated as heat in the body is less than the metabolic energy liberation In negative work e g walking downhill or resisting an external effect the supplied external energy is wholly or partly converted to heat in the muscles In this case the heat liberation in the body is that liberated by the metabolic processes plus the induced heat

It was shown (B Nielsen 1966 1969) that the plateau levels of deep body temperature during negative work was related to the oxygen uptake rather than to the total heat production while the sweat rate and skin circulation was a function of the total heat production (metabolic plus induced heat)

The purpose of the present study was to compare the effect of an environmental heat load on the circulatory and thermoregulatory reactions to positive and negative work

Methods

The cardiac output $\dot{Q}$ was measured (94 determinations) with the CO_2 rebreathing method (Defares 1958) as modified by Klausen (1965) and Klausen *et al.* (1968) and with the acetylene method as described by Asmussen and Nielsen (1952). This method was used alternately before or after the CO_2 method in 29 expts. at different environmental temperatures.

The blood flow in the working muscles was measured by the clearance method (Kety 1949) from m. vastus lateralis with ^{133}Xe as tracer (Lassen, Lindbjerg and Munck 1964).

Skin circulation was estimated from the changes in conductance (K) of the peripheral tissues (Burton 1934, Hardy 1937, Winslow *et al.* 1937). K was calculated for 15 min of exercise between 45 and 60 min of work as

$$K = \frac{H - E - S}{(\bar{T} - \bar{T})A_D} \text{ kcal/h} \times m^2 \times {}^\circ C$$

where

H — heat production
E — evaporative heat loss from the lungs (kcal/h)
S = storage of body heat = $(\bar{T}_{es} \times 0.65 + \bar{T} \times 0.35) \times$ body weight $\times$ specific heat of the body
A_D = DuBois area

The core temperatures were measured with copper constantan thermocouples at 3 depths (27, 22 and 17 cm) in the rectum (T) and just above the diaphragm in the esophagus (T_{es}) and recorded continuously (Kipp Micrograph BD 1). The position in esophagus was checked by x-ray in each subject.

Skin temperature (T) was measured at 15 locations (B. Nielsen and M. Nielsen 1962 and 1965). The average skin temperature ($\bar{T}$) was calculated by weighting the single measurements according to the size of the corresponding skin areas (Hardy and DuBois 1938).

The sweat evaporation was calculated from the weight loss measured on a Krogh balance (Krogh and Trolle 1936) as the difference between the weight loss determined from weighings after 5 and 60 min work and the respiratory losses due to evaporation and gas exchange.

The respiratory gas exchange was measured with the Douglas bag method and air samples were analyzed in duplicate according to Scholander (1947).

Positive work was performed on the Krogh ergometer with the saddle replaced by an armchair (M. Nielsen 1938) so that the subject worked with the legs in a horizontal position. The negative work consisted of resisting an external power transmitted through an induction clutch from an electromotor to the flywheel of the Krogh ergometer to the pedal (Petersen 1969).

The positive work (2[illegible]0 kpm/min, pos I) and negative work (1260 kpm/min, neg) was chosen so as to produce the same metabolic rate (approx. 0.8 l/min). Further a series of control experiments with positive work in which the total heat production was equal to that of the negative work were carried out (720 kpm/min, pos II) and a few experiments of positive work where the work rate and muscle tensions (work rate) equaled those in the negative experiments (pos III).

The heat production (H) was calculated as
H = M − W in the positive experiments and as
H = M + W in the negative experiments, where M is the metabolic energy liberation (4.9 kcal per l O_2) and W the external work in kcal/h.

The experiments were performed at 20, 30 and 35 and a few at 40 °C in a climatic chamber without forced air movements (B. Nielsen and M. Nielsen 1962). The humidity was not controlled but the water vapor pressure was close to 8–10 mm Hg in all experiments. The subjects were 3 male students.

subject	age yr	weight kg	height cm	A_D m^2
CM	24	76	183	1.97
[illegible]	22	[illegible]8	168	1.6[illegible]
JD	29	73	174	1.87

Procedure

The subject arrived in the morning after a light breakfast. He inserted the esophageal and rectal thermocouples and rested in the chair for [illegible] in the chamber at the selected temperature for 45 min. In the negative work experiments the induction clutch was calibrated and warmed up

TABLE I Average values of cardiac output measured in 3 subjects at different environmental temperatures n = number of determinations Ranges are shown in bracets Neg and pos I $\dot{V}_{O_2}$ = 0.8 l/min pos II $\dot{V}_{O_2}$ = 1.7 l/min

		20	n	30	n	35	n	40	n
LE	neg	9.5 (7.6—12.09)	5	12.8 (10.9—16.7)	5	11.4 (9.3—13.1)	4	11.3 (11.2—11.3)	2
	pos I	9.4 (8.1—10.2)	6	11.8 (11.6—11.9)	2	11.2 (8.6—14.6)	4	12.5 (10.3—14.6)	2
	pos II	16.4 (14.4—20.4)	5	17.5 (16.4—18.5)	2	16.2 (15.0—16.8)	4		
CM	neg	10.8 (8.8—13.7)	4	12.5 (8.9—15.0)	6	16.3 (9.7—20.1)	5		
	pos I	14.1 (12.2—15.6)	6	15.4 (11.3—18.7)	5	16.0 (13.0—21.6)	6		
	pos II	20.6 (15.4—28.4)	7	18.8 (11.5—22.3)	6	21.3 (17.9—23.7)	4		
JD	neg	11.1 (10.2—11.8)	4	12.8 (11.8—13.8)	2	15.1 (11.0—19.1)	2		
	pos I	9.2 (7.9—13.7)	3	11.6 (9.1—13.3)	4	13.5 (12.2—14.6)	4		
	pos II	19.4 (17.3—23.1)	4	17.0 (14.0—18.8)	5	17.4 (15.5—19.2)	5		

Just before starting to exercise the subject stepped over to the Krogh balance where he sat down for the weighing He returned to the ergometer and started either positive or negative work with n 3 min of the weighing The weighing was repeated during a 2 min pause after 20 min of work, and immediately after the approx 60 min work period

The metabolic rate was determined at 40 and 50 min of work, and just after each gas collection rebreathing in the Grollman bag for Q determination was started The heart rate was counted over the radial artery at 5 min intervals The skin temperatures were measured at 35 48 and 58 min of work

Four closely placed depots of 0.1 ml isotonic saline solution containing Xe was injected in m vastus lateralis or m rectus femoralis at the 5 and 45 min during a 20 s paus

Results

The average O_2 uptake for the 3 subjects in both the negative (neg) work and the lowest positive (pos I) work was about 0.8 l/min There was no difference in O_2 uptake between the experiments performed at different environmental temperatures

The cardiac output was the same at the same O_2 uptake in positive and negative work in all the subjects (t test) This is statistically significant at the 5 % level in all three subjects

The cardiac output (Q) increased in all 3 subjects with increasing environmental temperature between 20 and 35 C both in the experiments with light positive and in negative work However the scatter of the measurements were large and since Q of the 3 subjects are not parts of the same population their values cannot be pooled and the determinations at the different conditions are too few for a statistical mation of the increase The cardiac output results are presented in Table I

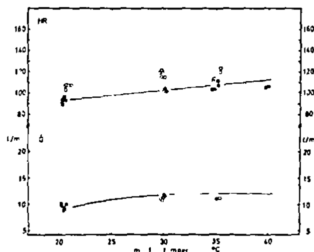


Fig 1 Heart rate and cardiac output for positive and negative work at the same oxygen uptake at different environmental temperatures Subj LF

○ pos I

● neg

The heart rate was approximately 10 beats/min higher in negative work than in positive work at the same oxygen uptake Fig 1 shows heart rate and $\dot{Q}$ in subject LF

No differences in the muscle blood flow expressed as the disappearance rate for Xe could be demonstrated between positive and negative work at the same metabolic rate (pos I and neg) Neither did changes in environmental temperature produce measurable changes in muscle blood flow However in experiments with higher metabolic rates (pos II and pos III) muscle blood flow was increased This will be discussed in more detail in a following paper (F Bonde Petersen B Nielsen and S L Nielsen)

The conductance (K) of the peripheral tissues for 2 subjects is plotted against environmental temperature in Fig 2 K increased with increasing environmental temperature For the same environmental temperature the conductance in positive work was about half the value of that obtained in negative work in the T_o range 20—40 C

Table II shows the calculated variations in skin blood flow The skin flow in l/min was calculated from conductance as $(K-K_{min}) \times A_D/60 \times$ specific heat of blood by assuming that blood is cooled from esophageal temperature to skin temperature as it passes to and from the skin Minimal conductance (K_{min}) for the subject was obtained from a plot relating K and $\bar{Sw}$ for $\bar{Sw}=0$ g/h Changes in conductance above minimum is regarded as being due to changes only in convective heat transfer (skin blood flow) neglecting the small change in conductive heat transfer The skin blood flow increases with increasing environmental temperature In negative work it is more than twice the value of that in pos I and a little higher than the values for pos II for all environmental conditions

Fig 3 shows the esophageal temperature in the 50—60th min in 3 conditions of work pos I neg and pos II for different environmental temperatures During negative

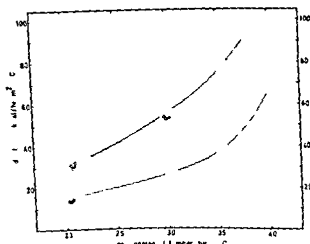


Fig 2. Conductance of the peripheral tissues for positive and negative work at the same oxygen uptake at different environmental temperatures 2 subjects

● LE, ▲ CM } pos I
○ LE, △ CM } neg

work T_{es} was slightly higher than during positive work at the same O_2 uptake (pos I) but lower than during positive work with the same heat production (pos II) The core temperatures were independent of the environmental temperatures in the studied range for the lowest work load and heat production (pos I) In the conditions with higher heat production (neg and pos II) the upper limit for thermal equilibrium was passed and the core temperature increased above the equilibrium level at the highest environmental temperatures The environmental temperature at which the temperature regulation broke down was lower for pos II than for neg

Mean values of average rectal temperature (average of measurements in 17 22 and 27 cm depth) $\bar{T}_{re}$ measured between 50—60th min of work (neg pos I and pos II) are shown in Table III for the 3 subjects $\bar{T}_{re}$ in neg deviates a little more than $\bar{T}_{es}$ in neg from the pos I values probably because it is influenced by the warm blood from the working muscles $\bar{T}_{re}$ in neg is however lower than $\bar{T}_{re}$ in pos II with the same heat production

TABLE II Calculated minimum skinblood flow l/min (cf p 25? * Values for subject JD are calculated using $\bar{T}$ instead of $\bar{T}_{es}$

		20	30	35	40
LE	neg	0 6	1 3	1 7	2 4
	pos I	0 1	0 4	0 6	1 6
	pos II	0 4	1 0	1 3	
CM	neg	0 7	1 5	? 3	
	pos I	0 1	0 5	0 8	
	pos II	0 5	1.3	1 4	
JD*	neg	0 9	1 1	1 6	
	pos I	0 1	0 5	0 7	
	pos II	0 4	1 0	[illegible]	

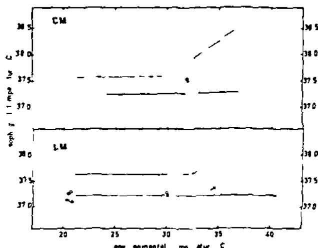


Fig 3 Deep esophageal temperature at 50—60 min of work at different environmental temperatures 2 subjects LE (below) and CM (above)

● positive work (pos I) 250 kpm/min
○ negative work (neg) 1250 kpm/min
+ positive work (pos II) 770 kpm/min

TABLE III The mean values of average rectal $\bar{T}_r$ (average of measurements in 17, 22 and 27 cm depth) and mean esophageal temperature $\bar{T}_{es}$ measured between the 50—60th min of work (neg, pos I and pos II) at different environmental temperatures 3 subjects n = number of experiments

			20	n	30	n	35	n	40	n
LE	neg	$\bar{T}$	37 46	(2)	37 61	(3)	37 51	(2)	38 14	(1)
		$\bar{T}_{es}$	37 27	(3)	37 37	(3)	37 35	(2)	37 97	(1)
	pos I	$\bar{T}$	37 14	(3)	37 24	(1)	37 30	(2)	37 35	(1)
		$\bar{T}_{es}$	37 18	(3)	37 23	(1)	37 24	(2)	37 13	(1)
	pos II	$\bar{T}$	37 78	(3)	37 76	(1)	37 92	(2)		
		$\bar{T}_{es}$	37 67	(3)	37 57	(1)	37 74	(2)		
CM	neg	$\bar{T}$	37 49	(2)	37 66	(2)	38 13	(3)		
		$\bar{T}_{es}$	37 41	(3)	37 50	(2)	38 05	(3)		
	pos I	$\bar{T}$	37 23	(4	37 36	(3	37 41	(3)		
		$\bar{T}_{es}$	37 22	(4	37 25	(3	37 27	(3)		
	pos II	$\bar{T}_r$	37 69	2	37 96	(3)	38 34	(3		
		$\bar{T}_{es}$	37 [illegible]7	(3	37 86	(3	38 46	(3		
JD	neg	$\bar{T}$	37 62	1)	37 61	1)	37 83	(2		
	pos I	$\bar{T}_{es}$	37 16	(?	37 48	2	37 56	(2		
	pos II	$\bar{T}$	37 69	3)	37 95	[illegible]	38 19	(2)		

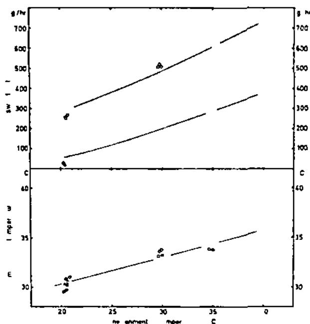


Fig 4 Above sweat rate below mean skin temperature during steady state of work at different environmental temperatures Subj LE

● positive work (pos I) 250 kpm/min

○ negative work (neg) 1250 kpm/min

— positive work (pos II) 720 kpm/min

The mean skin temperature increased linearly with increasing environmental temperature in the range studied independent of the type of work sweat rate and heat production (Fig 4)

The sweat rate (Sw) Fig 4 increased with increasing environmental temperature In conditions where the heat production approximately is the same (neg and pos II) the sweat rate was the same for the same environmental temperature (and consequently mean skin temperature) in spite of the differences in deep body temperature (Fig 3)

Discussion

During work the cardiac output increases as the oxygen requirement in the muscles rises Further the extra heat produced during work demands a certain blood flow to be removed and carried to the skin from where it is dissipated In our experiments with positive and negative work at the same oxygen uptake the oxygen requirements in the muscles and the muscle blood flows are presumably identical This assumption is at least not disproved by the ¹³³Xe clearance measurements which showed that the muscle blood flow was a function of the metabolic rate and was not measurably affected by the large intra muscular tensions in negative work or by variations in the environmental temperature (Bonde Petersen B Nielsen and Vanggaard 1970 Bonde Petersen B Nielsen and S L Nielsen)

The heat production, however, is about 3 times higher in the negative work and must represent an extra load on the circulatory system

The cardiac output was found to be the same in positive and negative work with the same oxygen uptakes This confirms the results for low oxygen uptakes at neutral temperatures (Thomson 1971) The increase in cardiac output which seems to occur in our experiments at 30—35 $\bar{T}_o$ however could not be reliably determined because the CO_2 method is not accurate enough to measure small differences

A minimal skin blood flow can be calculated from conductance if it is assumed that the blood is cooled from esophageal temperature to skin temperature as it passes to and from the skin (*cf* p 252) Any counter current heat exchange in the skin or between arteries and veins in the limbs will only increase the flow needed to carry the heat to the surface The calculated minimum skin blood flows increase approx *from 0 1 to 0 7 l/min in pos I and from 0 7 to 2 0 l/min* in negative work when $\bar{T}_o$ increased from 20 —35 (Table II)

Since total cardiac output and muscle blood flow as mentioned before most probably are the same in positive (pos I) and negative work at the same environ mental temperature it can be concluded that the higher skin blood flow needed in negative work even at 20 $\bar{T}_o$ must be compensated for by a decreased flow in other organs These are presumably the splanchnic organs as found in exercise of increas ing severity (Rowell *et al* 1964 Clausen and Trap Jensen 1970) and with external heat loads (Radigan and Robinson 1949 Rowell *et al* 1965)

The thermoregulatory reactions to an increased environmental temperature during negative work were found to be the same as in positive work 1) the average skin temperature $\bar{T}_s$ increased with increasing temperature (Fig 4) independently of type of exercise and work load (B Nielsen 1969) 2) the sweat rate changed with environmental temperature and skin temperature in relation to the heat production *(neg and pos II Fig 4) in spite of the lower core temperature* ($\bar{T}_{es}$ *and* $\bar{T}_{re}$) *during* negative work But the upper limit for thermal equilibrium was reduced in negative work compared to positive work at the same O_2 uptake (and $\bar{T}_e$ and $\bar{T}_s$) This is most probably explained by the higher skin blood flow during negative work As discussed above the visceral blood flow is presumably already reduced at 20 in negative work compared to positive work at the same O_2 uptake With the extra external head loads at 30—35 and 40 C the recruitment of blood therefore sooner reaches a limit and the core temperature is forced to rise above the equilibrium value at neutral temperatures In pos II with the same *heat* production as in the negative work the upper limit for thermal equilibrium is even lower than in the negative work (Fig 3) The skin circulation is nearly the same as in the negative work (Table II) but total cardiac output and the oxygen demand are higher Apparently the blood reserve available for heat transport in pos II is reduced in spite of the higher Q This assumption is supported by the findings of *e g* Rowell *et al* 1964 that the hepatic blood flow is reduced with increasing O_2 uptake

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Fluid Transfer between Blood and Tissues during Exercise

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Abstract

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During 6 min exercise on a bicycle ergometer the volume of the leg in normal males increased so as to indicate after correction for increased regional blood volume an average transcapillary fluid loss into the leg muscles of 19 31 and 45 ml/kg tissue at light (300 kpm/min) moderate (900 kpm/min) and heavy (1200—1500 kpm/min) work load The total fluid loss into the active muscle mass was calculated to comprise about 1100 ml during heavy work Since the concomitant decrease of plasma volume was 600 ml it follows that some 500 ml of fluid must have entered the circulatory system during the work The study indicated that this compensatory fluid gain was accomplished by absorption of extravascular fluid from inactive tissues and partly caused by osmosis resulting from work induced arterial hyperosmolality (average increase 22 mOsm/kg H_2O) Fluid absorption from inactive tissues was studied in experimental animals during exercise and in resting humans during arterial hyperosmolality produced by intravenous hypertonic infusions The investigations suggested that at least half of the fluid gain to the circulatory system in heavy exercise could be ascribed to the increased arterial osmolality and the remainder to a reflex decrease of capillary pressure

Exercise is associated with a net transcapillary movement of fluid into the active muscles mainly caused by regional tissue hyperosmolality (osmosis) and to some extent by increased capillary hydrostatic pressure (filtration) (Mellander *et al* 1967 Lundvall 1972) The extent of this fluid accumulation has previously been determined during work with a small muscle group (lower leg muscles of the cat) The fluid accumulation was related to the work intensity and most rapid in early stages of exercise reaching a plateau with time During heavy work the accumulated fluid could amount to 15 to 20 per cent of the resting tissue volume in 15 min (Jacobsson and Kjellmer 1964 Lundvall 1972)

If fluid accumulated to the same extent in all active muscles during whole body exercise it can be deduced that the fluid loss from the circulatory system would exceed the entire plasma volume in a relatively short period of time It is well known that in man plasma volume decreases by at most 15—20 per cent or by some 600 ml during heavy work with large muscle groups (e g Åstrand *et al* 1964 Ekelund and Holmgren 1964 Hartley *et al* 1970 This may imply either that fluid accumula

tion is much more limited when large than small muscle groups are active and no greater than the actual plasma volume decrease or if more pronounced that fluid must be transferred into the circulatory system for instance from the extravascular space of inactive tissues The present study in which the fluid accumulation in the active muscle mass and the change of plasma volume were estimated during short term bicycle exercise in man suggested that the latter train of events takes place Studies on experimental animals indicated that fluid absorption from inactive tissues was caused by transcapillary hydrostatic as well as osmotic forces the latter established by a significantly increased arterial osmolality apparently resulting from delivery of osmoles from the exercising muscles A preliminary report has been published previously (Lundvall *et al* 1970)

Methods

Experiments in man Observations were made on a total of 19 healthy lean males mean age 27 years (range 20—42) mean b w 75 kg (range 60—95) and mean height 183 cm (range 170—193)

The experiments started in the morning and the subjects who were instructed to avoid physical exercise before the investigation rested in the supine position for at least 1/2 h before any experimental procedures were undertaken The subjects were in the supine position during the experiments except when standing for the leg volume measurements (see below) and when sitting during exercise (see below)

Graded exercise was performed on an electrically braked bicycle ergometer (Elema Schonander Sweden) Observations were made of heart rate changes of plasma volume osmolality in arterial and venous blood and fluid accumulation in the leg muscles Changes of plasma volume and arterial osmolality were also studied in resting subjects in response to intravenous hypertonic infusions

In experiments in which blood sampling or infusions were made polyethene catheters were inserted as described by Berneus *et al* (1954) Arterial blood was sampled from the left brachial artery (used for plasma volume and osmolality determinations) and venous blood via a catheter inserted into the left femoral vein below the inguinal ligament and advanced some 10 cm distally into a deep vein (used for osmolality determinations in venous effluent from muscle) Infusions and injections were made via a catheter introduced into a suitable arm vein on the right side and advanced to the subclavian vein Occasionally several such catheters were inserted

Plasma volume was determined using [1] I radio-iodinated human serum albumin (RIHSA 0.05 μc/kg body weight AB Atomenergi Sweden) and changes of plasma volume by the hemoglobin method (*cf* Holmgren 1956 Fricke 1965 Poortmans 1971) RIHSA was given iv at the beginning of the experiment During a 30 min period following the injection of RISHA and before any other experimental procedures were undertaken repeated arterial blood samples were withdrawn for well counter measurement of the radioactivity of plasma "Zero-time plasma radioactivity was determined by extrapolation on semi log graph and plasma volume was calculated using appropriately diluted standard samples prepared from the radioactive solution injected Total hemoglobin was calculated from simultaneously determined values for hemoglobin concentration (cyanomethemoglobin method) and hematocrit (microcentrifugation technique corrected for trapped plasma) assuming that the ratio between whole body and peripheral hematocrit was 0 91 (Blendis *et al* 1970) During the following experimental procedures arterial hemoglobin concentration and hematocrit were measured repeatedly and using the initial value for total hemoglobin total blood volume and red blood cell mass were calculated Plasma volumes were obtained by subtraction.

Plasma osmolality was determined by thermistor cryoscopy (Osmometer 31 LAS Advanced Instruments Inc USA) and each sample was measured twice If occasionally different readings were obtained the mean value was used Readings in repetitive measurements on osmolar standards deviated at most by 1 5 mOsm/kg H_2O from the true value

All blood samples were taken after discarding the "dead space fluid volumes of the catheters and collected in test tubes containing a small amount of dry heparin (not influencing the osmolality of the sample) and kept in ice water Plasma was separated by centrifuging within 30 min after withdrawal.

Intravenous infusions (usually 200 ml) were made in 20 min or less using motor driven syringes. Each subject received an isotonic control infusion and, after an interval of at least 30 min, a hypertonic infusion, both infusions being identical as to rate and volume. The isotonic solutions used were glucose (0.055 g/ml) or sodium chloride (0.009 g/ml) and the hypertonic solutions were glucose (0.3 or 0.6 g/ml) or mannitol (0.15 or 0.20 g/ml). The solutions were sterile and pyrogen free. No significant untoward effects of the infusions were noted.

The total blood loss due to sampling during an experiment (duration 2.5—4 h) was at most 150 ml. No correction was made for this gradual blood loss.

Changes of leg volume were measured using a water filled (≈34 °C) lucite boot designed to enclose the subject's right leg either up to the knee region or up to the mid thigh region. Measurements were made on the subject in upright posture and the positioning of the leg in the boot and the load on either foot could be kept virtually identical in repetitive measurements after training. A horizontal slit opening in the front wall of the boot a few cm below its upper edge permitted quick drainage of excess water above this level when an outflow tube connected to the slit was opened. Drainage was facilitated by the addition of a small amount of a surface tension decreasing agent to the water in the boot.

By the following procedures blood volume in the resting leg segment could be reduced and kept virtually constant during the measurements of leg volume in the erect posture. Before each measurement an attendant elevated the leg of the supine subject to 45° angle during 15 s to drain the regional capacitance vessels in a standardized manner. This procedure caused as standardized a drainage as more prolonged elevation or as caused by the application of external pressure (> 70 mm Hg) on the tissue via an inflated surgical leg air splint. After 15 s of elevation of the leg a tourniquet cuff (18 cm wide) applied around and fixed to the upper part of the thigh was inflated to about 280 mm Hg, a level reached in less than 1 s by means of a connected pressure container. The subject then rose to the erect posture and placed his right leg into the boot filled to an appropriate level with water. When the subject was standing still in correct position, excess water was drained down to the level of the slit opening through the outlet tube. The outlet was then closed and the subject returned to the supine position. The cuff was deflated and the water amount adhered to his leg determined from the increased weight of a towel used for wiping. After correction for this water volume a baseline for further leg volume determinations had been established. The volumetric measurement could be repeated as soon as the cuff induced reactive hyperemia had vanished. At each successive measurement a standard amount of water was poured into the boot. The same amount (measured with a graduated cylinder) would be drained upon opening the outlet if leg volume was the same as in the preceding determination. A larger amount would be drained if leg volume had increased, and vice versa. In this way changes of leg volume could be determined.

Each experiment started by making about 10 such determinations in the resting control state. After the subject had been accustomed to the measurement procedures, control leg volume varied only slightly in consecutive measurements. Baseline tissue volume in the last 4 control measurements thus varied from its mean value by ±4.6 ml S.D. (±1.2 ml/kg soft tissue) on the average, or by ±0.12 per cent of the total soft tissue of the segment. When control leg volume had been established (the absolute figure being obtained at the end of the experiment, see below) the subject performed exercise of 6 min duration on the bicycle ergometer (300—900 or 1200—1500 kpm/min). Heart rate was determined repeatedly by auscultation or from ECG recordings. The subject lay down in supine and relaxed position immediately after the work, the leg was elevated, the thigh cuff pressure applied as described and the change of leg volume determined (in less than 30 s after cessation of work). As will be reported, leg volume invariably increased after exercise above the resting control value and then declined with time. This decline was followed until the control value had been reached.

The aim of the volumetric measurements was to estimate the fluid amount accumulated in the extravascular space of exercising muscle. Although the above described good reproducibility in the volumetric measurements indicated that in the control state regional blood volume was kept virtually constant, it was conceivable that the exercise vasodilatation could lead to some regional blood volume increase despite the measures taken to minimize blood pooling. The extent of such a blood volume increase during work in all probability is correlated to the degree of the evoked exercise hyperemia (active and passive blood volume increase, cf. Mellander and Johansson 1968). Its magnitude during heavy exercise might therefore be revealed by determining the increase of leg volume immediately after prolonged ischemia, which also causes a virtually maximal hyperemic response but, unlike heavy work, hardly any extravascular fluid accumulation, since reactive hyperemia is not associated with any significant regional tissues hyperosmolality (unpublished observations, cf. also Discussion). Ischemia was produced for 5 min in the resting supine subjects by the application of suprasystolic pressure in the thigh cuff. After this period the subject rose to erect posture and the cuff pressure was

released When the reactive hyperemia was fully developed (as judged by the change of skin colour in the foot) the subject lay down quickly again and then underwent the same procedures as described above for leg volume measurement after exercise Another method for estimation of regional blood volume changes not only after heavy but also after lower work loads will be described in Results section

At the end of each experiment when the leg volume was restored to the control value the total volume of the tissue segment was measured by determining the water amount required to fill the boot precisely up to the level of the outflow opening Soft tissue volume (≈ weight) of the leg was derived by subtracting the volume of the bone A figure for the latter was obtained by measuring the volume of the corresponding bone segment on a medium-sized skeleton Since virtually all extravascular fluid accumulation in exercise is confined to skeletal muscle tissue (see Discussion) of which there is little in the foot the volume of the foot was subtracted from the volume of the total leg segment in the boot whereas no correction was made for non muscular soft tissues of the leg itself

9 subjects performed heavy work (1200—1500 kpm/min) which was virtually maximal for these individuals Some of these subjects performed exercise at lower work intensities also and the different experiments were then performed at intervals of several days.

Experiments in cats Experiments were performed on a total of 15 cats anesthetized intravenously with a mixture of α-chloralose (50 mg/kg) and urethane (100 mg/kg) Fluid absorption from the extravascular space of the resting right foreleg was studied during experimentally induced heavy exercise of both hind limbs To measure fluid absorption the foreleg was enclosed in a water filled temperature regulated (34 C) plethysmograph (*cf* Mellander 1960) After heparinization venous outflow of blood from the region was measured by a drop-recorder unit connected to the cognate axillary vein other venous drainage being prevented by occlusion Blood was returned to the animal via the left jugular vein Venous outflow pressure was adjusted so as to produce an isovolumetric state in the control situation Arterial pressure was monitored from the contralateral axillary artery Arterial plasma osmolality was determined repetitively in samples withdrawn from a T tube in the arterial pressure cannula. Exercise of the lower leg muscles was produced by bilateral stimulation of the distal ends of the severed sciatic nerves (4 imp/sec 0 5 msec 2—3 volts) The body was firmly fixed by placing a vertical steal core between the two legs against the pubical bone and stretching the hindlegs via snares around the paws By this means and by fixation of the right foreleg in the plethysmograph changes of foreleg volume during hindleg exercise could be adequately recorded without an interference of motion of the limb

Possible changes of regional blood volume could be separated from net transcapillary fluid fluxes in the foreleg by concomitant external monitoring the radioactivity of the blood over the region (51Cr method for ref see Mellander and Johansson 1968) Net transcapillary fluid fluxes were calculated for foreleg soft tissue the weight of the latter determined by dissection at the end of the experiment (volume changes during the course of the experiment being taken into account) Changes of foreleg volume during hindleg exercise were observed in one series of expts in animals with intact sympatho-adrenal system and in another series after regional sympathectomy and bilateral ligation of the adrenals In a third series of expts fluid absorption from the foreleg was observed during hypertonic glucose (15—25 %) infusions administered via the left axillary vein

In Results section below spread of data is expressed as SEM both for the human and animal experiments

Results

Experiments in man Fig 1 shows for a representative experiment the changes of tissue volume (ml/kg soft tissue) observed in the lower leg immediately after six min of exercise on the bicycle ergometer (1200 kpm/min) and in the following period of rest In this subject exercise led to an initial tissue volume increase of 60 ml/kg muscle and about an hour elapsed before the tissue volume had returned to the control level

In spite of the measures taken to maintain a constant regional blood content during the measurements (see Methods) some blood volume increase must have occurred during the hyperemic phase in the early postexercise period The ext such a regional blood volume increase immediately after heavy exercise was

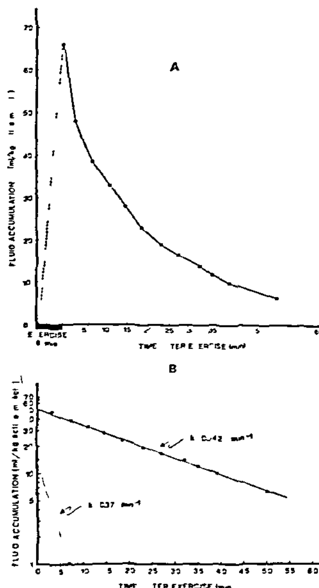


Fig. 1 Changes of volume in the lower leg immediately after 6 min of exercise (bicycle ergometer 1200 kpm/min final heart rate 195/min and in the following period of rest in an adult male subject (linear plot panel A and semilogarithmic plot panel B By compartment analysis of data in panel B a slow component reflecting the disappearance of fluid accumulated in the extravascular space and a fast component reflecting the decay of increased blood volume could be distinguished

by determining the tissue volume increase immediately after a period of prolonged regional ischemia see Methods Such estimations were made on 7 of the subjects who performed heavy exercise These experiments indicated that leg blood volume initially increased by an average of 12 4 ± 1 0ъ ml kg soft tissue

When the observed changes in tissue volume were plotted semilogarithmically (panel B of Fig 1 it was found that the plotted values fell along an approximately straight line except for those in the initial period after cessation of work. This finding suggests an approximately monoexponential disappearance of the fluid accumulated in the extravascular space at a rate given by the drawn line (method of

least squares rate constant 0 042 min⁻¹) Analysis of the data in panel B suggested that in addition to this slow component an initial fast component was present (rate constant 0 37 min⁻¹) The intercepts of the two component lines on the y axis represent initial volumes of 52 and 14 ml/kg soft tissue respectively for the slow and fast components The latter value agrees with the regional blood volume increase estimated in the above mentioned ischemia experiments 12 4 ml/kg Furthermore the fast component of the leg volume decrease curve coincides in time with the hyperemic phase This conclusion is based on the fact that postexercise hyperemia is known to vanish in an approximately exponential manner (Dornhorst and Whelan 1953 Hyman 1968), and after heavy work with a rate constant of similar magnitude to that of the above mentioned fast component (Lundvall 1972) These considerations make it reasonable to assume that the fast and slow components of the leg volume decrease represent the disappearance of pooled blood and of accumulated extravascular fluid respectively

By the described graphical method estimations of the blood volume increase and of the extravascular fluid accumulation could be made not only after heavy work but also in the experiments with moderate and light exercise Fig 2 shows the collected results concerning the extravascular fluid accumulation (ml/kg soft tissue of the lower leg) immediately after graded exercise and the subsequent fluid disappearance A work load of 1200 kpm/min was nearly maximal for the subjects (mean heart rate 180±4/min) whereas 900 kpm/min may be considered as moderate exercise (heart rate 153±8/min) and 300 kpm/min as light work (heart rate 95±5/min) It can be seen from the figure that there is a relation between the extent of fluid accumulation immediately after exercise and the work intensity the average values being about 45 ml/kg at heavy exercise 31 ml/kg at moderate exercise and 19 ml/kg at light exercise The time required for the accumulated fluid to disappear from the muscles was longer the greater the work load and it could exceed 1 hour at heavy work

The results from the experiments in which the lower leg muscle as well as part of the thigh muscles were included in the measurements were quite similar to those depicted in Fig 2 This suggests that the given figures for fluid accumulation may be considered representative for all leg muscles which on the average comprised 20 kg (determined by volume displacement measurements) and in all probability for several other muscle groups as well The data therefore indicate that quite large fluid volumes ($\approx$ 1100 ml) escape from the circulatory system into the active muscles during heavy bicycle work (see Discussion)

The concomitant plasma volume decrease during heavy exercise was determined in 4 subjects At the end of a 6 min exercise period the plasma volume decrease averaged 610 ± 16 ml or 17 per cent of control plasma volume It thus appears that the fluid loss during exercise can be much larger than the net decrease of plasma volume indicating that a compensatory fluid transfer into the circulatory system takes place The studies reported below were performed in an attempt to reveal mechanisms responsible for such plasma fluid replacement

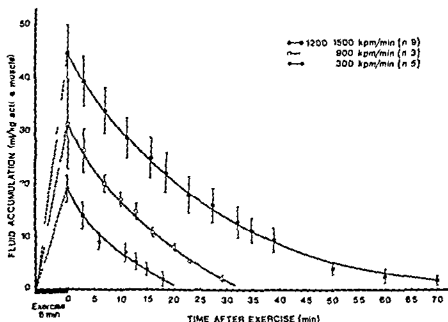


Fig 2 Collected data on the extravascular fluid accumulation in the human lower leg muscles immediately after 6 min of graded bicycle work and on the fluid disappearance in the post exercise resting period

By repetitive blood sampling changes of osmolality in the arterial plasma were followed during graded exercise of 6 to 10 min duration in 5 subjects (11 expts) and concomitantly in the femoral vein in 2 subjects (7 expts) These experiments showed that arterial osmolality began to rise above the resting control level shortly after the onset of work to approach plateau values within 4 to 6 min The degree of arterial hyperosmolality was related to the work load and reached an average value of 22 ± 2.0 mOsm/kg H_2O ($n = 5$) above the control level (mean control value 286 ± 1.8 mOsm/kg H_2O) during heavy work (virtually maximal for the subjects) The arterial hyperosmolality seemed to be somewhat less pronounced if work was repeated after a period of rest The osmolar change in the arterial blood was clearly preceded by hyperosmolality in the venous effluent from active muscle and it reached higher levels than in the arterial blood The veno-arterial osmolar difference was greatest in early periods of work and declined with time Apparently the arterial hyperosmolality is caused by delivery of osmoles from the exercising muscles

It is conceivable that perfusion of inactive tissues with hypertonic blood during exercise could cause an osmotic absorption of extravascular fluid into the circulatory system As a first crude approach to this problem changes of plasma volume were determined during arterial plasma hyperosmolality in resting subjects ($n = 5$) produced by i v infusions of hypertonic glucose or mannitol solutions On the average these infusions lasted for about 10 min and caused a gradually increased

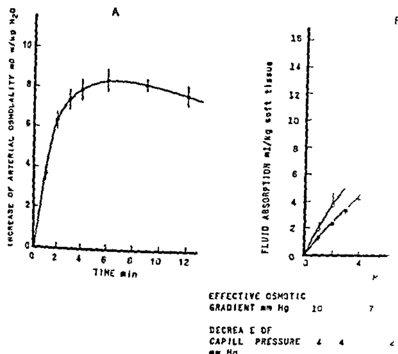


Fig 3 Panel A Increase of arterial osmolality above the control level d r i g r Panel B Cumulative fluid absorption from resting foreleg in the cat during i i The upper curve refers to experiments (n = 10) with intact sympatho-adrena lower curve to experiments (n = 11) in which the influence of the s mpatl a on th fl id bsorption was eliminated

arterial osmolality which at the end of the infusions exceeded control osmolality by 14±14 mOsm/kg H O Concomitantly there was a gradual increase of plasma volume which averaged 313±45 ml or 10 per cent of plasma volume at the end of the infusions (In 2 expts, initial plasma volume was not determined but estimated from body weight (*e g* Flemming 1955) The value for plasma volume increase was obtained after correction for the slight effect of the infused volume *per se* (control isotonic infusions) About 40 per cent of this plasma volume expansion was still p esent half an hour after the cessation of the hypertonic infusion These experiments show that arterial hyperosmolality caused by small molecules (as in exercise see Discuss n) can lead to considerable fluid absorption from inactive tissues

Experiments in cats The mechanisms responsible for transcapillary fluid absorption f om resting tissues during exercise were elucidated in some more detail in anesthetized cats In these expts net transcapillary fluid absorption in the resting foreleg was recorded continuously during concomitant heavy exercise with the lower leg muscles in both hindlimbs As can be seen from Fig 3 (panel A) arterial osmolality rose by an average of 8 mOsm/kg H O above the resting control level in

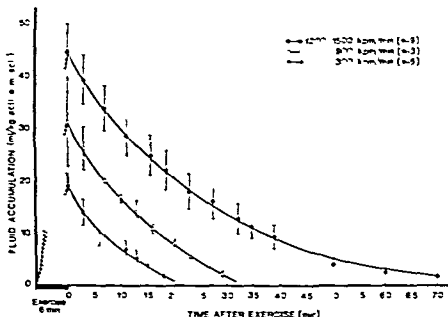


Fig. 2. Collected data on the extravascular fluid accumulation in the human lower leg muscles immediately after 6 min of graded bicycle work, and on the fluid disappearance in the post-exercise resting period.

By repetitive blood sampling changes of osmolality in the arterial plasma were followed during graded exercise of 6 to 10 min duration in 5 subjects (11 expts.) and concomitantly in the femoral vein in 2 subjects (7 expts.). These experiments showed that arterial osmolality began to rise above the resting control level shortly after the onset of work to approach plateau values within 4 to 6 min. The degree of arterial hyperosmolality was related to the work load and reached an average value of 22 ± 2.0 mOsm/kg H_2O (n = 5) above the control level (mean control value 286 ± 1.8 mOsm/kg H_2O) during heavy work (virtually maximal for the subjects). The arterial hyperosmolality seemed to be somewhat less pronounced if work was repeated after a period of rest. The osmolar change in the arterial blood was clearly preceded by hyperosmolality in the venous effluent from active muscle and it reached higher levels than in the arterial blood. The veno-arterial osmolar difference was greatest in early periods of work and declined with time. Apparently the arterial hyperosmolality is caused by delivery of osmoles from the exercising muscles.

It is conceivable that perfusion of inactive tissues with hypertonic blood during exercise could cause an osmotic absorption of extravascular fluid into the circulatory system. As a first crude approach to this problem, changes of plasma volume were determined during arterial plasma hyperosmolality in resting subjects (n = 5) produced by i.v. infusions of hypertonic glucose or mannitol solutions. On the average these infusions lasted for about 10 min and caused a gradually increased

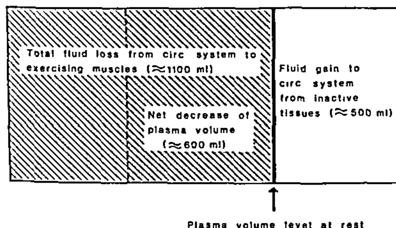


Fig 4 Schematic illustration of the fluid shifts between the intra and extravascular compartments during short term heavy bicycle exercise in male subjects By osmosis and filtration about 1100 ml of plasma fluid is lost to the active muscles As an effect of work induced arterial hyperosmolality and reflex adrenergic mechanisms some 500 ml of extravascular fluid is simultaneously absorbed from inactive tissues The net decrease of plasma volume is thus limited to about 600 ml

tively the capillary pressure rise in upright posture by lowering regional venous pressure (*e g* Folkow *et al* 1970 1971) These observations indicate that only a fraction of the fluid accumulation in the leg muscles observed in the present study during short term work can be ascribed to the hydrostatic load per se The major part in all probability is caused by osmosis resulting from increased muscle tissue osmolality as shown to be the case in the cat (see Lundvall 1972) It is conceivable therefore that fluid loss occurred also into other muscles (some 14 kg in the present subjects) than those of the legs and that in some the loss was almost as large as in the leg muscles as least during heavy bicycle work However in order not to over estimate the total fluid loss in exercise only 5 kg of these muscles or a total of 25 kg were used for the extrapolation Thus the present data (*cf* Fig 2) indicated that the total fluid loss into active muscle was in the order of 1100 ml during heavy work 800 ml during moderate work and 500 ml during light work

Plasma volume did not decrease by more than 600 ml during 6 min of virtually maximal exercise a figure in good agreement with other studies (*e g* Ekelund and Holmgren 1964) and therefore a rapid gain to the circulatory system of some 500 ml of fluid must be assumed to have occurred Fluid gain by metabolic production of water can be considered negligible in short term exercise (for ref see Åstrand and Rodahl 1970) and therefore fluid must have entered the circulatory system from the extravascular space of inactive tissues Since fluid loss via kidneys skin and lungs is insignificant in this short period of time the fluid shifts between the intra and extravascular compartments during short term heavy bicycle exercise can be visualized as in the schematic illustration of Fig 4

Fluid absorption from inactive tissues was more directly evidenced by different experimental approaches in cat and man which also indicated that it was evoked by osmolar and reflex adrenergic control mechanisms. The former was coupled to work induced arterial hyperosmolality of considerable magnitude. Circumstantial evidence from hypertonic infusion experiments in man and from the cat studies could be taken to indicate that at least half of the fluid gain (Fig. 3) was caused by osmosis and the remainder by a reflex decrease of capillary hydrostatic pressure. The work induced arterial hyperosmolality, which mainly seems caused by small molecules (lactate, sodium, potassium etc., see Lundvall 1972) thus can lead to considerable fluid absorption even if the transcapillary osmotic reflection coefficients for these substances in all likelihood are less than unity (perhaps < 0.3, *cf.* Lundvall 1972). The plasma volume reduction per se must in addition lead to some increase of the plasma protein concentration and thereby reinforce the osmotic fluid absorption process created by smaller molecules. Fluid absorption from inactive tissues due to a reflex decrease of capillary hydrostatic pressure would be an effect of the well known increase of sympathetic activity in exercise and compatible with what is known to occur in other situations of increased sympathetic discharge, for instance during moderate hemorrhage (Mellander and Öberg 1967).

In the present experiments, in which large muscle groups were active, fluid accumulation was only about 50 per cent of that reported during short term heavy work in the cat performed with a small muscle group (Lundvall 1972). This may partly be ascribed to the fact that in the present experiments there was a recirculation of blood with considerably raised osmolality to active muscle which must have decreased the transcapillary osmotic gradient from tissue to blood, hence limiting the osmotic fluid loss into the exercising muscles. The discrepancy further can be attributed to some drainage of extravascular fluid from muscle to the circulatory system via the lymph vessels during the bicycle work. Such drainage was prevented in the above mentioned cat experiments by ligation of the regional lymph vessels. Lymph drainage from muscle mainly occurs during limb movements but may be almost negligible at rest (Jacobsson and Kjellmer 1964). This may help to explain that the disappearance of the accumulated fluid in the present study followed an approximately mono-exponential course in the resting postexercise period (Fig. 1), since fluid removal then mainly may have occurred by transcapillary absorption.

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From the Institute of Physiology University of Oslo Norway

The Effects of Intraperitoneally and Intravenously Administered Pentobarbitone Anesthesia on Pulmonary and Splanchnic Blood Volumes in Rats An Evaluation Carried out before and after Standardized Blood Losses

By

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Abstract

Aarseth P *The effects of intraperitoneally and intravenously administered pentobarbitone anesthesia on pulmonary and splanchnic blood volumes in rats An evaluation carried out before and after standardized blood losses* Acta physiol scand 1972 *85* 270—276

Pulmonary and splanchnic blood volumes have been examined with the use of suitable radioactive isotopes in rats anesthetized with either intraperitoneally or intravenously administered pentobarbitone (30 mg/kg b w) The blood volumes in the two vascular areas were evaluated both in non bled rats and in animals which had been exposed to a 12 % or a 24 % reduction of the total blood volume Organ blood content was examined after sudden circulatory arrest which was achieved by rapid freezing of the whole animal in liquid nitrogen. I p anesthetized non bled rats had a smaller splanchnic blood volume and a larger pulmonary blood volume than the i v anesthetized rats The large lung blood volume of i p anesthetized rats was markedly reduced upon a 12 % blood loss A 24 % blood loss caused moderate additional pulmonary blood volume reductions Also in the i v anesthetized animals the lung blood volume was relatively more reduced during a small bleeding than during a large one A 12 % blood loss here caused only small volume changes in the splanchnic vascular bed whereas a marked blood volume reduction occurred in this area after a 24 blood loss It appears that the pulmonary vascular bed of the rat is very sensitive to changes in total blood volume and that it easily alters its capacitance in parallel with total blood volume changes

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Various types of investigations have shown that certain sections of the cardiovascular system can easily change their volume when this is needed Both the pulmonary vascular bed and the splanchnic vasculature are known to exert such depot functions The absolute and relative extent of the depot functions of these vascular areas and also the mechanisms behind them have previously been examined in rats in a series of investigations (Aarseth 1970 Aarseth 1971 a and b Aarseth and Piene 1972) When animals were exposed to a sudden blood loss both these vascular areas could markedly reduce their volume but the relative degree of blood volume reduc

tion appeared to depend to a large extent on the type of anesthesia used Aarseth and Piene (1972) thus found that the splanchnic vascular bed was a more important blood depot than the lung vessels in bled ether anesthetized rats However hardly any volume reduction occurred in the splanchnic vessels of bled rats which had been anesthetized by an i p injection of pentobarbitone In this situation the lung vessels acted as the more important blood depot

In the present investigation the effects of various types of anesthesia upon the blood depot functions of the pulmonary and splanchnic areas have been further explored The effect of a larger bleeding was thus compared in animals anesthetized with either an i v injection or an i p injection of pentobarbitone

Erythrocytes and plasma albumin which had been labelled by radioisotopes were injected i v and sudden circulatory arrest was then achieved by freezing the intact animal in liquid nitrogen The blood content of various organs was found from their content of isotopes Results from bled animals were compared with results obtained in non bled control animals

It was found that the different methods of anesthesia under investigation did not qualitatively disturb the depot function of the lung vessels or the splanchnic vessels The initial blood volumes in these vascular beds were however influenced by the method of anesthesia and the depot functions could thereby be quantitatively changed

Methods

Animal groups Male albino Wistar rats weighing 208—255 g were used They had free access to food and water until the experiments started The animals were divided into two main groups according to whether they should receive pentobarbitone i v or i p These main groups were again divided in subgroups so that each main group consisted of one control group of non bled animals and one or two groups of bled animals

Anesthesia All animals were anesthetized with pentobarbitone (Nembutal® Abbott) 30 mg/kg b w The animals in one of the main groups received an intraperitoneal injections of Nembutal diluted 1 4 in saline (2 ml/kg b w) The right femoral vein was then cannulated and 0 2 ml of heparin diluted in saline were injected i v In the other main group a catheter was introduced into the femoral vein during l ght ether anesthesia Mean exposure time to ether was 7 min ±2 (S D) Pentobarbitone (diluted 1 2 in saline containing heparin 800 I U / ml) were then given i v The injection was given as 2 shots the second dose injected 10—15 min after the first one The total volume injected i v to each animal in this group was 0 21—0 25m l

Labelling of blood with radioisotopes ^{51}Cr labelled rat erythrocytes were mixed with an qual volume of saline contain ng ^{12} I labelled human serum album n (Institutt for atomenergi Kjeller Norway) About 0 2 ml of this mixture were injected i v 40 min after the start of pentobarbitone anesthesia (For details see Aarseth 1970)

Bleeding was performed through the femoral catheter by a constant rate withdrawal pump and started 5 min after the injection of the isotope mixture The bleeding lasted for 5 min and the pump rate u ed was calculated so as to give a blood loss of either 12 % or 24 % of the estimated total blood volume

Blood sampl ng In the control animals a blood sample of 0 2 ml was withdrawn 8 min afte the injection of isotopes in order to find the hematocrit and the isotope concentrat on in the blood In animals which were bled about 12 % during 5 m n it has been sho n that there is no decrease in hematocrit or in isotope concentration during the bleeding itself (Aarseth 1970) In an mals bled 12 the shed blood as thus used fo measurements of hematocrit and blood isotope concentrations In animals which are bled about 24 % both the hematocrit and the plasma album n concentration decrease during the bleed ng procedure (Aarseth 1970) In animals bled this much a small separate blood sample was therefore withdrawn immed tely after the bleeding had ended

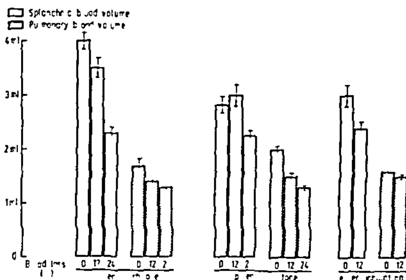


Fig 1 Pulmonary blood volume and splanchnic blood volume in differently treated groups of rats The effect on organ blood content of a blood loss amounting to 12 % or 24 % of total blood volume is evaluated in animals anesthetized in 3 different ways i v injected pentobarbitone i p injected pentobarbitone and ether inhalation Bars on top represent ±S E Some of the results used have been published previously (Aarseth 1970 Aarseth and Piene 1972)

Discussion

Previous experiments on rats have shown that during i p anesthesia lung blood volume was markedly reduced upon a small (12 %) blood loss while there was relatively little further volume reduction upon a larger (24 %) bleeding (Aarseth 1970) During the same form of anesthesia the splanchnic blood volume showed no reduction upon a small bleeding (Aarseth and Piene 1972) The reaction of this vascular bed to a larger blood loss was then not tested

Rats anesthetized in a different way by ether inhalation showed a different reaction to a blood loss Here a 12 % bleeding caused a much more marked volume reduction of the splanchnic than of the lung vascular bed (Aarseth and Piene 1972) This quantitative difference from animals anesthetized by an i p injection of pentobarbitone might have several reasons the pentobarbitone *per se* might *e g* cause the difference or the i p route of administration might be responsible for the different pattern of blood mobilization

In order to test these possibilities blood mobilization was examined in animals anesthetized by intravenously injected pentobarbitone The same pentobarbitone dose (30 mg/kg b w) was used Also the same fluid volume was injected i v to these animals as to the animals with i p anesthesia Although the duration of anesthesia is dependent on the route of administration the same time schedule was followed in the two types of experiments with circulatory arrest 50 min after the initiation of pentobarbitone anesthesia The pattern of blood pressure depression

found in the present experiments was very similar to that previously found after i p injection of pentobarbitone (Aarseth 1970) with almost no depressive effect left after 50 min A 12 % bleeding was also found to be tolerated equally well and without any reduction in systemic arterial pressure

The present experiments showed that the way in which blood is distributed between the two important vascular beds of the lungs and of the splanchnic area is markedly influenced by the type of anesthesia used Thus the control animals anesthetized by an i v pentobarbitone injection did not have the same organ blood content as had the non bled control animal anesthetized by i p injections (Table I and II) The splanchnic blood volume was much smaller in the latter group whereas the pulmonary blood volume was larger One possible explanation for this difference could be that i p pentobarbitone injection causes some blood to be expelled from the splanchnic area leading to a shift towards a greater pulmonary blood volume

The smallest lung blood volume of non bled rats has been found in ether anesthetized animals (Aarseth and Piene 1972) The sympathetic nervous system influences the capacitance vessel of the lung (Aarseth 1971 a) and ether inhalation is known to release a sympathomimetic effect (Price 1960) It could therefore be that the non bled ether anesthetized animals had got their lung blood volume reduced through this mechanism of the anesthetic itself (Fig 1)

On a 12 % blood loss the patterns of blood mobilization differed from animals with i v pentobarbitone anesthesia to animals with i p pentobarbitone anesthesia. This qualitative difference was however apparently connected to the different prebleeding organ blood content in these groups of animals (Table I and II Fig 1) I p anesthetized animals with a large prebleeding pulmonary blood volume mobilized much blood (25 %) from this vascular organ bed very little from the splanchnic area In the i v anesthetized animal with a more moderate prebleeding pulmonary blood volume there was a mean lung blood volume reduction of only 16 % a mean splanchnic blood volume reduction of 14 % (Fig 1) In both groups the splanchnic blood volume was markedly reduced after a blood loss of 24 % (Fig 1) It can also be seen from Fig 1 that the two groups of differently anesthetized animals had their lung and splanchnic blood volumes reduced down to the same level after a 24 % blood loss

In an isolated cat hind limb muscle preparation Lundgren Lundvall and Mellander (1964) found that the sympathetic nervous discharge which led to maximal constriction of capacitance vessels occured after a relativly small blood loss It is very likely therefore that the reduction seen in splanchnic and pulmonary blood volumes after a 24 % blood loss involves the maximal active constriction achievable in the capacitance vessels of these areas

The net weight of the lung tissue was found by subtracting from the total weight of the lung the calculated content of blood Changes of the mean net tissue weight have been described and discussed previously (Aarseth 1970 Aarseth 1971 b) Leakage of plasma albumin and thereby overestimation of blood volume in the

lung was not detected It was thus concluded that changes of net tissue weight reflected changes in lung extravascular water content From Table I and II can be seen that this content is reduced when the total blood volume is reduced The extra vascular water content in the lungs was however larger in the non bled animals anesthetized by i p injections than in the i v anesthetized animals This may be related to some degree of pooling of blood in the pulmonary vascular bed in the former group of animals

The present results seem to support the conclusion that the pulmonary vascular bed has an important function as a blood depot This depot is apparently easily drawn upon during a blood loss especially when the prebleeding lung blood volume is large The degree of reduction in volume of the splanchnic vascular area upon a moderate bleeding is depending on the type of anesthesia used After a large blood loss splanchnic blood volume was always found to be markedly reduced regardless of type of anesthesia used

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From the Institute of Physiology University of Oslo Norway

Dehydration-Induced Reductions in Total Blood Volume and in Pulmonary Blood Volume in Rats

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Abstract

AARSETH P and D KLLG *Dehydration induced reductions in total blood volume and in pulmonary blood volume in rats* Acta physiol scand 1972 85 277—282

Groups of rats were exposed to water deprivation and/or food deprivation for 24 h periods The degree of dehydration obtained was evaluated by weight examinations of the differently treated rats The plasma and erythrocyte volumes were estimated by the use of iv injected isotopes It was found that on water deprivation the intravascular fluid compartment was *reduced relatively more than the total body water content The blood content in the lungs* was estimated from the organ content of isotopes after the rats had been rapidly frozen in liquid nitrogen When dehydration had caused an 8 % reduction in the total blood volume the pulmonary blood volume was found to be reduced by 30 The pulmonary vascular compartment thus appears to function as an important blood depot in this connection

The relationship between the intravascular and the extravascular fluid compartments is not clear under all conditions The present experiments have been carried out in order to see how much the plasma volume and the total body water content were reduced during dehydration in rats Also we wanted to know if the pulmonary vasculature was more prone to dehydration induced volume reductions than other sections of the vascular bed

The results indicate that the plasma compartment became relatively more reduced than the total body water content A very large proportion of this blood volume reduction took place in the lungs

Methods

Animals Male albino rats of one strain and within a weight range of 21?—?00 g were used They were initially kept for 1—5 days in one large cage in our animal house During this period they had free access to water and dry pellet food The day before the experiment each animal was given a light ether anesthesia in order to get the exact body weight Individual animals were then placed in small cages where they were divided at random into four groups exposed to different water and food schemes The characteristics of the groups are given Table I

TABLE I. Four groups of rats were kept at different food and water schemes for 20—24 h according to this Table. The + and — in each column indicate whether the animals had been offered or denied food or water. The number of animals in each group and their mean b.w. are also given.

	Group			
	A	B	C	D
n	12	12	16	18
Body weight g ± S.D.	244 ± 13	247 ± 13	239 ± 13	247 ± 16
Food supply	+	+	—	—
Water supply	+	—	+	—

The animals were kept in these cages and on these schemes for 20—24 h. They were then given 30 mg/kg b.w. of pentobarbitone intraperitoneally (Nembutal® Abbott diluted 1 : 4 in saline) and again weighed. Their right femoral vein was cannulated and 0.2 ml of a mixture of ^{51}Cr labelled rat erythrocytes and ^{125}I human serum albumin (Institutt for Atomenergi, Kjeller, Norway) was given i.v. 5—7 min later a blood sample was withdrawn and a few min later the animal was immersed in liquid nitrogen. The lungs and a sample from the left thigh muscle were removed from each frozen rat. The measured hematocrit and the radioactivity in the injected isotope mixture, in the blood sample and in the tissue samples were used for calculation of total erythrocyte and plasma volumes as well as for calculation of the tissue erythrocyte and plasma volumes. The calculation procedures have previously been described in detail (Aarseth 1970).

Hematocrit was measured with the use of an International microcapillary centrifuge (Model MD). Tissue hematocrit was calculated from the estimated erythrocyte and plasma volumes in the tissues.

Serum proteins were estimated by the method of Kalckar (1947), measuring optical density at 260 and 280 nm. In each group serum samples were obtained by taking blood by heart puncture from 3 animals. These animals were not used for any other measurements.

Wilcoxons two sample test was used for evaluation of significance of differences between groups.

Results

In Fig. 1 is shown the weight changes in the different groups of animals. The control group (group A) had a small weight gain, while the 3 other groups all showed large weight losses during their last day. The difference between groups C and D, which both had been without food, must be due to lack of water in group D. This difference amounts to 4.5 g ($p < 0.001$) or 1.6 % of b.w. The weight difference between groups A and B (which also differed only as regards water supply) was 21 g, and thus much larger than the weight difference between groups C and D.

In order to compare plasma and erythrocyte volumes in the different groups these volumes were calculated as ml/100 g initial b.w. All experimental groups showed changes both in their plasma and in their erythrocyte volume when compared to the values in the control group (A) (Fig. 2). In group B, which had been without water, the plasma volume was reduced by 0.3 ml/100 g b.w. ($p = 0.04$). This reduction corresponds to about 9 % of the plasma volume in the control group. The erythrocyte volume *increased, however, to almost the same extent, so that the total* blood volume was about the same in the 2 groups.

Group C, which did get water but no food, had about the same plasma volume and erythrocyte volume as group B. In the animals in group D, which had got

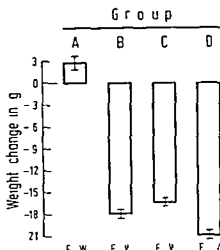


Fig 1 Change in b w of rats which for the last 20—24 h had been exposed to different food and water schemes F+ and W+ indicate free access to food and water respectively F– and W– indicate that there was no access to food and water during the 20—24 h For more detailed de scription of the groups see Table I Bars represent 2 S E

neither food nor water for 20—24 h the plasma volume was further reduced to 2 99 ml/100 g b w The difference between groups C and D as regards plasma volume was 0 32 ml/100 g b w ($p = 0\ 035$) Since there was no further increase in erythrocyte volume also the total blood volume in group D was reduced when compared with the other groups (Fig 2)

The hematocrit and the serum protein concentration found in the animals from the 4 groups are listed in Table II The hematocrit increased in all experimental groups when compared with the control group A ($p < 0\ 01$) The serum protein concentration increased in the 2 dehydrated groups (B and D $p = 0\ 05$) but not in group C which had had access to water but not to food

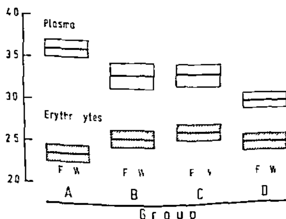


Fig 2 Plasma volumes and erythrocyt olumes (ml/100 g initial b in 4 group of rats wh ch fo the last 20—24 h had been on d fferent food and water schem Descript ons of the group are gi en in Fig 1 and in Method Table I The volumes gi n repr ent the m an alues ±S D

TABLE II. Hematocrit values in blood from the femoral vein and serum protein concentration in blood obtained by heart puncture. The hematocrit values (mean ± S.D.) are obtained from the animals in the 4 ordinary experimental groups (see Table I). Each of the mean serum protein values is obtained from 3 animals treated like those in the ordinary groups but not used for any other measurements than this one (see Methods)

	Group			
	A	B	C	D
Hematocrit	45 ± 1.2	51.2 ± 1.5	47.9 ± 1.8	49.4 ± 3.1
Serum protein (mg/ml)	44 (40–49)	89 (71–117)	48 (43–54)	57 (55–60)

TABLE III. Pulmonary blood volume, pulmonary hematocrit and skeletal muscle blood content for animals which had been without food and water for 20–24 h (group D) and for animals in a control group (A) with free access to food and water. Mean values ± S.D.

	Group		Difference
	A	D	
Pulmonary blood volume (ml)	2.0 ± 0.5	1.4 ± 0.5	0.6 p = 0.003
Pulmonary hematocrit as % of large vessel hematocrit	65 ± 14	78 ± 9	13 p = 0.008
Muscle blood content (μl/g tissue)	27 ± 10	21 ± 7	6 p = 0.03

In Table III are compared the pulmonary blood volume and the muscle blood content in the control group A and the group D which had got neither food nor water. (The results obtained in groups B and C lay between those found in groups A and D.) In group D the pulmonary blood volume had decreased from 2 to 1.4 ml while the pulmonary hematocrit had increased from 65 to 78 % of large vessel hematocrit. The muscle blood content decreased from 27 to 21 μl/g in the dehydrated and fasted animals.

Discussion

In the rat both the daily intake of food and the daily metabolic activity markedly influence the body weight. It is therefore difficult to estimate the degree of dehydration by observing weight changes only. Since dehydration will also most probably interfere with eating, one cannot for this purpose simply compare for example groups A and B.

It can thus be seen from Fig. 1 that animals which had free access to food but which were deprived of water for 24 h showed a weight reduction of 21 g. When one compares two groups of animals (C and D) which were both deprived of food but where one of them only had access to water for the last 24 h, they showed a weight difference of only 4.5 g. The degree of real dehydration was probably of the

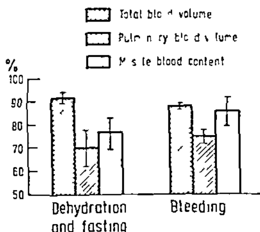


Fig 3 *Degree of reduction in pulmonary* blood volume and in skeletal muscle blood content in rats where a) total blood volume has been reduced by 8 % due to deprivation of food and water for 20—24 h and b) total blood volume has been reduced acutely by 12 % through a bleeding The values are expressed as % of the values found in control groups Bars on top represent ±S E (The bleeding experiments have been published elsewhere Aarseth 1970)

same order in groups B and D and hence represents about 4 5 ml of body water If the total water content in the animal amounts to 65 % of b w then 3 % of this water is lost during a 20—24 h dehydration period

During such a dehydration period the plasma volume in the rat is reduced by about 10 % both in the animals which had got food and in the food deprived animals (Fig 2) The intravascular compartment is thus reduced relatively more than other fluid compartments in the body during dehydration This is in accordance with the findings of Gregersen and Bullock (1933) and of Myhre and Robinson (1971) They measured plasma volume in man after dehydration due to water deprivation or due to excessive sweating Also lack of food for 20—24 h seems to reduce the plasma volume in the rat When group C in which the animals had had water but no food was compared with the control group the plasma volume was found to be reduced by 0 3 ml/100 g b w In accordance with this the hematocrit had increased There was however no increase in serum protein concentration in group C (Table II) The plasma volume reduction in fasted animals may thus primarily be due to a loss of plasma protein with reduced colloidosmotic pressure as a consequence The metabolic rate relative to body weight is 10—15 times higher in rat than in man and it is conceivable that plasma proteins may have been metabolized to some degree during a 24 h period of fasting in rats

Some compensatory increase in erythrocyte volume took place when the plasma volume was decreased due to lack of food alone or to a combined lack of food and water or due to lack of water alone Still in the food and water deprived group D total blood volume was 1 1 ml less than in the control group It has previously been shown in pentobarbitone anesthetized rats that when total blood volume is acutely reduced by a bleeding then the volume of the pulmonary vascular bed is relatively more reduced than that of other vascular bed (Aarseth 1970 Aarseth and Piene 1972) At the same time pulmonary hematocrit increased considerably in the bled group From Table III it can be seen that the pulmonary blood volu

relatively markedly reduced and the pulmonary hematocrit increased also during the dehydration induced reduction in total blood volume In Fig 3 the results of the present experiments are compared with previous results from rats bled 12 % of their total blood volume (Aarseth 1970) In the present dehydration experiments the compensatory reduction in lung blood content on reduction in total blood volume is even more marked than the one seen in the bleeding experiments This further brings to the attention the role of the pulmonary vascular bed as a buffering blood depot

This work has been supported by a grant from The Norwegian Council on Cardiovascular Diseases as well as by grants given to the Institute of Physiology from The Nansen Foundation.

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From the Department of Anatomy University of Helsinki Finland

Ultrastructural Changes Induced by Estrogen in the Adrenergic Nerves of the Rabbit Myometrium

By

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The female reproductive tract is partially innervated by short adrenergic neurons (Owman and Sjostrand 1966) which differ functionally as well as anatomically from the ordinary long adrenergic neurons One of these differences seems to be the ability of the short neurons to react to estrogen administration (Sjoberg 1967 1968 Kanerva *et al* 1972) After 17 β estradiol treatment both the density of the effective innervation and the transmitter content of individual axons seemed to be increased The aim of the present preliminary account is to describe the corresponding changes at fine structural level

Adult albino rabbits were injected daily with 17 β estradiol (1 μg/kg) for 10—14 days The uterus oviduct and vagina were prepared for several procedures The tissue pieces for electron microscopy were fixed in 3 % ice cold potassium permanganate as described by Hokfelt (1968) The ultrathin sections were viewed and photographed with Philips EM 300 electronmicroscope operated at 40 kV Tissues from untreated adult preovulatory rabbits served as controls

In the present account the main attention was directed to the preterminal nerve fibre and the terminal axons originating from it The effects of the estrogen at the ultrastructural level on the nerves were the following (1) The dimensions of the axons were increased up to the double from the controls The small granular vesicles containing the transmitter were more evenly dispersed over the enlarged cross sectional area of the axons (Fig 2—4) The diameter of the axons reached that of the varicosities and there were thus less variations in the thickness of the nerves (2) The amount of neurotubuli increased The newly appeared tubuli were short and coiled and contained often electron opaque material The shape of these membranous structures was often irregular and the term tubule could hardly be applied for them (Fig 3) The tubuli were found also within the varicosities although they were most abundant along the course of the axon In extreme views the whole cross sectional surface of the axon was covered with randomly coiling neurotubuli (Fig 4) (3) The proportion of large vesicles increased from the normal 2—3 % to 5—7 % Furthermore large agranular vesicles of the same size (diameter 700—1200 Å) as the large granular vesicles were found (Fig 4) The large vesicles were occasionally in close contact with the neurotubuli At this position they could often be considered as distentions of the plasma membrane of the neurotubuli

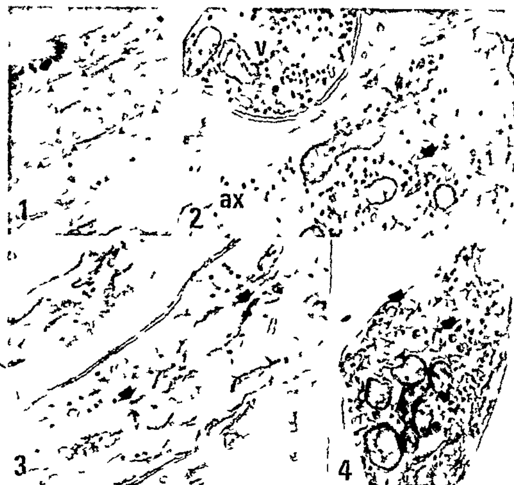


Fig 1 Longitudinal section of a preterminal nerve fibre from the myometrium of a control animal Two adrenergic and one non adrenergic axon run parallelly separated by the cytoplasm of Schwann cell (triangles) Note the agranular small vesicles of the nonadrenergic axon × 27 400

Fig 2 Distended axonal enlargement (V) and an wide axon (ax) in the preterminal nerve fibre after estrogen treatment The axonal enlargement does not differ much from the controls but the axon (ax) is extremely distended Note the agranular vesicles (arrow) × 27 400

Fig 3 Longitudinal section of 2 distended axons after estrogen treatment Note the presence of large amount of neurotubuli and the electron dense content of some of them (arrows) × 27 400

Fig 4 Cross section of an axon showing extreme degree of the changes produced by estrogen Richly coiling neurotubuli are present Note the large agranular vesicles (arrows) × 27 400

In addition to the changes listed above there might have been changes also in the number of the small granular vesicles and in the neuromuscular junctions However since careful serial sectioning has not yet been performed accurate data on these details will be presented later *The axo axonal contacts between the adrenergic*

and nonadrenergic axons described previously by Hervonen and Kanerva (1972 a b) seemed to remain unaltered

The fluorescence microscopy of the autonomic nerves of the rabbit uterus and oviduct revealed that the fluorescence of individual axons was more intensive after estrogen treatment Furthermore the axons appeared swollen and distended (Sjoberg 1968) and the general density of the network of fluorescent nerves was increased All these changes suggested increase in the transmitter content of the adrenergic axons The findings listed above might be due to the increased capacity to store noradrenaline The increase in the amount of neurotubuli and the electron opaque material found within the tubuli might be signs of formation of new storage sites for noradrenaline A more detailed description of the changes produced by the steroid hormones in the autonomic nerves of the female reproductive tract will be published in near future

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Acta physiol scand 1972 85 286—288

From the Institute of Physiology University of Helsinki Finland

Respiratory Arrest and Bradycardia during Anterolateral Diencephalic Stimulation in the Chicken

By

P V Kotilainen and P T S Putkonen

Our experiments were aimed at elucidating circulatory and respiratory effects of electrical brain stimulation at a diencephalic area which in a previous study (Putkonen 1967) gave a complex stimulation syndrome in freely moving chicken This predominantly trophotrope syndrome—in terms of Hess (1947)—consisted of crouching and locomotor inhibition tonic ruffling of the feathers miosis and cardiac deceleration Apnea was probably present but was missed because respiration was not recorded and its visual observation was hindered by the intensely ruffled plumage

The present experiments were performed on 10 anesthetized (Nembutal® 40—60 mg/kg i m.) white Leghorns Blood pressure and heart rate were measured from a cannula in the ischiadic artery with an electromanometer Respiratory movements were monitored with a thoracometric transducer Records were obtained with an electroencephalogram at paper speeds of 2 mm/s (Fig 1—2) or 15 mm/s for heart rate counting Mostly the birds breathed naturally but in some the trachea was cannulated to test the effects of O_2 or airway occlusion Thin monopolar steel electrodes were used in stereotaxic exploration of the brain The electrode tracts (2—4 per bird) were oriented according to the atlas of van Tienhoven and Juhasz (1962) (Fig 2) Stimuli were rectangular cathodal 2 ms pulses at a 50/s Intensities ranged for 200 to 600 μA Histological verification guided by small electrolytic lesions and prussian blue markings followed each experiment

Of 380 stimulated points mainly in the lateral diencephalon and basal forebrain an apneic response was obtained from 60 (16 %) polypneic patterns often with rhythmic vocalization—from 89 (23 %) and no respiratory changes from 231 (61 %) The typical apneic response (Fig 1 A) was associated with progressive bradycardia a fall in blood pressure ruffling of the feathers and miosis The points for the apneic

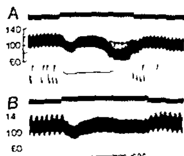


Fig 1 Blood pressure and respiration during 400 μA stimulation at a diencephalic point when the bird is breathing air (A) and during tracheal infusion of O_2 (B) Inspiratory apnea and hypotension is seen in both cases while bradycardia develops only in A and not in B in spite of a longer apnea under O_2 Stimulation indicated on the time scale (1 s divisions) Blood pressure in mm Hg Inspiration is upwards in the respirogram

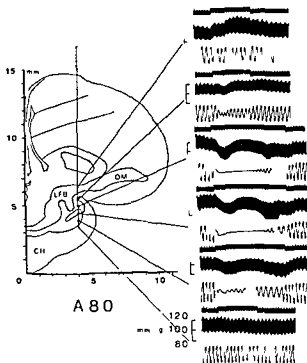


Fig 2 Circulatory and respiratory effects of 400 μA stimulation at successive points along an electrode tract (coordinates Ant 8 0 Lat 4 0) projected in a transverse section of the stereotaxic atlas Maximal apnea and bradycardia in the lateral diencephalon (Depth 4 5) Note hypertensive effect in the occipito-mesencephalic tract (OM) and lack of response in the chiasma (CHI) AR = archistriatum *LFB = lateral forebrain* bundle

response were clustered anterior to and around the nucleus rotundus but not within it (Fig 2) The area can be delineated by stereotaxic coordinates Anterior 6 5—8 5 Lateral 2 5—4 0 Horizontal 3 0—6 5 The pattern and the localization of the response conform with results from unanesthetized birds (Putkonen 1967)

Hypoxia was an important factor for the development of bradycardia since it was almost abolished by tracheal infusion of 100 % O during stimulation (Fig 1 B) In a representative test a bradycardic response of 29 % declined to 9 % under O whereas tracheal occlusion during stimulation increased the response to 67 % Air way closure alone was clearly less efficient

Feigl and Folkow (1962) elicited a diving response electrically from the medial mesencephalon of anesthetized ducks It consisted of apnea bradycardia muscular vasoconstriction and hypertension in contrast to the hypotensive effect of our di encephalic stimulation Thus our results resemble more the hypoventilatory and hypotensive effects from the trophotrope endophylactic sector in the lateral thala mus and subthalamus of the cat (Hess 1947) In one mesencephalic plunge how ever we hit some points giving apneic bradycardia with hypertension The localiza tion fitted with Feigl s and Folkow s (1962) area A for the diving response The best effect came from the quinto-frontal tract which may be significant in view of the claimed involvement of trigeminal input in the diving response

The importance of hypoxia and hypercapnia to the diving response was shown by the above authors. In monkeys bradycardia during amygdala stimulation is secondary to hypoventilation hypoxia and can be abolished by O_2 (Reis and McHugh 1968). In mammals respiratory inhibition is a common finding from many limbic structures (e.g. Kada 1951, Bergstrom, Hirvonen and Karlsson 1966). In our study we did not get apneas from the archistriatum (amygdala) nor from any other telencephalic sites. Vediaev (1963), who has a few respiration inhibitory points within our diencephalic area, however, reports some short apneas from the hyperstriatum in unanesthetized chronically implanted doves.

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Acta physiol scand 1972 85 289—296

From the Department of Physiology University of Goteborg Sweden

Effective Transmitter Concentrations in the Rat Portal Vein as Reflected by Neurogenic Potentiation of Responses to Exogenous Noradrenaline

By

Bengt Ljung and Goran Wennergren

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Abstract

Ljung B and G Wennergren *Effective transmitter concentrations in the rat portal vein as reflected by neurogenic potentiation of responses to exogenous noradrenaline* Acta physiol scand 1972 *85* 289—296

In the rat portal vein exogenous noradrenaline (NA) and the transmitter released during nerve activity seem to react with the same set of α receptors located on muscle cells close to the nerve terminals Very shortlasting high transmitter concentration peaks have been calculated to occur at the receptors for each nerve impulse In the present study an indirect approach was utilized to evaluate to what extent these peak concentrations in view of their short duration are effective in activating the smooth muscle Electrical field stimulation of the adrenergic nerve supply of the rat portal vein was applied during fully developed responses to graded concentrations of exogenous NA Transmural stimulation at 2—32 imp/s was found to increase the established responses to exogenous NA in concentrations up to 10^{-5} M These effects were abolished by tetrodotoxin The findings imply that the high shortlasting NA concentrations which occur at the α receptors for each nerve impulse lead to high transient degrees of receptor occupancy during nerve activity The effective transmitter concentrations over a wide range of impulse frequencies thus seem to approach the levels of NA which produce maximum responses when applied exogenously

In the rat portal vein the adrenergic nerve terminals are arranged in a main two dimensional ground plexus and do not penetrate into the muscle tissue of the media (Johansson *et al* 1970) which in principle is similar to the arrangement found in most blood vessels Exogenously administered noradrenaline (NA) and the transmitter released during nerve activity functionally activate the same population of adrenergic α receptors (Johansson *et al* 1972) and these receptors seem to be located predominantly on muscle cells in the vicinity of the nerve terminals (Johansson *et al* 1970) Calculations on the distribution of the endogenous NA in the [illegible] during repetitive nerve stimulation show that for each impulse high but [illegible] lasting transmitter concentration peaks occur at the muscle cells next to [illegible] of NA release (Ljung 1970 Johansson *et al* 1972) It is not possible to [illegible]

directly to what extent these transient peaks are able to activate the receptors of the effector cells, i.e. to measure the "effective transmitter concentration" during nerve activity. Indirectly this concentration could be reflected by the degree to which an established response to a certain concentration of exogenous NA can be potentiated by postganglionic nerve stimulation at a given impulse rate. A neurogenic increase in contractile activity during exposure to exogenous NA would indicate that the released transmitter significantly raises the degree of receptor occupancy. In the present study neurogenic increases in contractile activity of the isolated rat portal vein have been determined at different rates of nerve activity for graded concentrations of exogenous NA. The results are discussed with regard to their implications on the effective transmitter concentrations during nerve activity.

Methods

The experiments were performed on isolated portal vein preparations from rats (250 g) of the Sprague Dawley strain. The portal vein was isolated after the animal had been killed by a blow on the neck. Two preparations were mounted in a 30 ml organ bath containing a modified Krebs solution for isometric recording of contractile activity. The preparations were allowed to accommodate in the bath for 1 h before the actual experiment was started. The mean force developed over each 1 min period was quantified by electronical integration. Neurogenic responses were induced by electrical field stimulation (0.8 ms, 15 V) at different frequencies and responses to exogenous NA were obtained by injections of the amine directly into the bath (for details see Johansson et al. 1970).

Each NA concentration was applied for 2 periods of 4 min duration. Transmural stimulation of one preparation was performed during the third minute of the first period of NA exposure, and during the second NA administration the other preparation was stimulated also during the third minute. Thus each vessel was twice exposed to each NA concentration and during one of these periods electrical field stimulation was applied. The interval between all NA injections was 15 min or more, which allowed complete recovery of the normal spontaneous activity.

The mean force developed during the third minute of the NA exposure period was expressed in per cent of that developed during the second minute. Consequently, for each Na concentration in the bath two per cent values were obtained, one when the preparation was exposed only to exogenous NA and one when field stimulation was also applied. The difference between these values was taken to represent the "neurogenic increase" in contractile activity.

The composition of the Krebs solution used was in mM: NaCl 122, KCl 4.73, $NaHCO_3$ 15.5, KH_2PO_4 1.19, $MgCl_2$ 1.19, $CaCl_2$ 2.49, glucose 11.5 and $CaNa_2$ versenate 0.026. It was continuously bubbled with 4 % CO_2 in O_2. Temperature was kept at 37 °C.

Drugs: *l*-noradrenaline bitartrate (Norexadrin® conc., Astra); Tetrodotoxin (Sankyo Company Ltd, Tokyo, Japan). Fresh stock solutions were diluted with saline immediately prior to injection into the bath.

Results

Fig. 1 shows recordings of mechanical activity from one experiment where the nerves were stimulated at 8 imp/s. The typical spontaneous activity and the contractile responses to increasing concentrations of exogenous NA are seen in the left panel. The right panel shows the responses to the same NA concentrations but in addition the intramural adrenergic nerves were activated by electrical field stimulation at a frequency of 8 imp/s during the third minute of NA exposure. The neural influence manifests itself as an increase in contractile activity clearly seen when stimulation is applied during responses to low and moderate concentrations

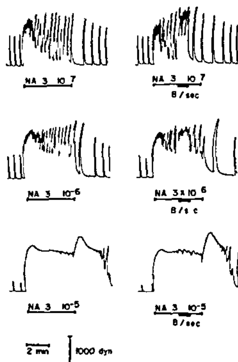


Fig 1 Tracings of isometric force developed by an isolated preparation of the rat portal vein Left panel shows spontaneous activity and excitatory responses to exogenous NA (M) Right panel shows the effect of electrical field stimulation at 8 imp/s applied during the third min of exogenous NA exposure Note clear potentiation in upper and middle recordings and small potentiation obtained during exposure to NA 3 10^{5} M

of exogenous NA (upper and middle recordings) but at a high NA concentration 3 10^{5} M (lower recording) the contractile response added by field stimulation is small In the experiment shown the nerve stimulation during the third minute of exposure to NA in concentrations of 3 10^{7} 3 10^{6} and 3 10 M resulted in mean force developments which were increased by 117 32 and 4 per cent respectively

In each experiment one frequency (2 4 8 16 or 32 imp/s) was applied and exogenous NA concentrations from 10^{7} M to 3 10^{5} M were administered Fig 2 summarizes the values for the neurogenic increase in contractile activity (for calculations see Methods) obtained in the present series of experiments at the different impulse rates and exogenous NA concentrations When field stimulation was superimposed on a NA concentration of 10^{7} M the increase in contractile activity induced by nerve activity was dependent on the impulse frequency and reached its maximum at 16 imp/s With higher concentrations of exogenous NA the addition in contractile response induced by nerve stimulation diminished At a concentration of 10^{5} M stimulation with 2 or 4 imp/s hardly potentiated the response obtained with exogenous NA but higher stimulation frequencies were still able to increase the mean force At 3 10 M only a negligible effect of nerve stimulation was recorded

Control experiments were performed according to the same experimental schedule after the preparation had been exposed to tetrodotoxin (7 10 g/ml) No potentia

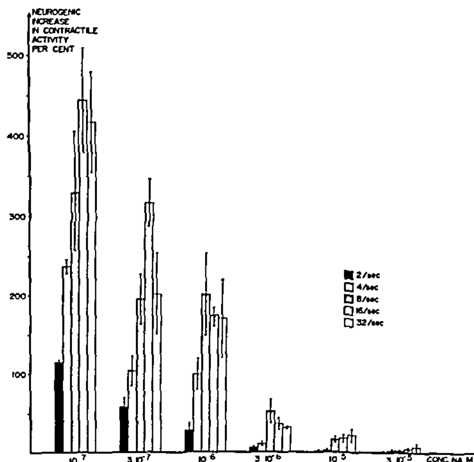


Fig 2 Potentiation of responses to graded concentration of exogenous NA induced by electrical field stimulation (0.8 ms 15 V) at the impulse frequencies indicated Each bar represents mean value (±S E) from four portal vein preparations For method of calculation see Methods

tion of the responses to exogenous NA was then obtained with electrical field stimulation Thus field stimulation with the stimulus parameters used did not activate the smooth muscle directly even in the presence of exogenous NA

Discussion

The mechanisms of the neuromuscular transmission in blood vessels have been found to vary with differences in anatomical and functional properties in different parts of the vascular tree Further the fact that the terminal adrenergic nerve elements do not penetrate into the smooth muscle mass in most blood vessels profoundly contrasts to the situation in most non vascular tissues The neuro effector system of the portal vein is of interest since it seems to offer a model for *in vitro* studies of vasomotor control of single unit vascular muscle (*cf* Ljung 1970)

Previous experiments have indicated that in the rat portal vein the α receptors engaged in responses to exogenous NA are located close to the nerve terminals (Johansson *et al* 1970) and that essentially the same set of receptors become engaged whether the agonist NA is released from the nerve terminals or exogenously administered Studies of NA release (Haggendal *et al* 1970) and calculations on transmitter distribution in the tissue have shown that very shortlasting high NA concentration peaks ($> 10^{5}$ M) should prevail at the receptors for each nerve impulse (Ljung 1970 Johansson *et al* 1972) Recent theoretical considerations based on the characteristics of the NA dose response and the frequency response curves of the portal vein suggest that each of the transmitter peaks may lead to transient high or nearly complete α receptor saturation (Johansson *et al* 1972)

In the present study transmural stimulation at 2—32 imp/s was found to potentiate responses to exogenous NA in concentrations in the bath up to 10^{5} M As no significant potentiation resulted from electrical field stimulation after the nerve function had been abolished by tetrodotoxin the potentiation obtained by field stimulation is to be attributed to the action of the released transmitter For each nerve impulse the peak concentration of the transmitter will transiently be added to the exogenous NA level which prevails at the receptors In view of the characteristics of the concentration effect curve for exogenous NA (see Johansson *et al* 1970 Fig 10 Johansson *et al* 1972) the sum of these concentrations must considerably exceed the exogenous one for a potentiation to occur Therefore the results imply that the amplitude of the transmitter concentration peaks in the rat portal vein resulting from nerve stimulation over a wide range of frequencies are equal to or greater than the local level of exogenous NA for bath concentrations up to the order of 10 M

To interpret this finding it is essential to consider what exogenous NA levels can be expected to occur at the α receptors as compared to the given external concentrations The versenate present in the Krebs solution prevents NA decomposition and the bath concentration can be considered constant during the 4 min exposure period Diffusion into the extracellular space of this thin walled preparation occurs quite rapidly (*cf* Arvill Johansson and Jonsson 1969) and for all practical purposes the exogenous NA concentration in the region of the receptors should have reached a steady state during the third minute of NA exposure *i e* when the nerves were stimulated The neuronal uptake mechanism however is capable of reducing the NA concentration at the receptors in the rat portal vein (Johansson *et al* 1970) Pharmacological inhibition of the uptake mechanism was not utilized in these experiments since uptake blocking drugs may also interfere with NA release and with smooth muscle activity (Haggendal *et al* 1972) Consequently the local concentrations must have been lower than in the surrounding bath fluid at low and moderate exogenous NA levels but since the uptake mechanism is a saturable process (*e g* Langer and Trendelenburg 1969) such a concentration difference is not expected to occur at NA concentrations of 10^{5} M and higher It thus seems likely that the exogenous NA concentration at the receptors when nerve stimulation no longer

potentiates the contractile response amounts to 10 —3 10 M which then would represent the effective transmitter concentration during nerve activity under these experimental conditions This value corresponds to the concentration of exogenous NA which leads to the maximum adrenergic response of the rat portal vein (Johansson *et al* 1970)

The distribution of the released transmitter undoubtedly becomes altered with increasing exogenous NA concentrations The presence of exogenous NA in the neuromuscular gap changes diffusion and as a consequence, the transmitter concentration profile must be of reduced amplitude and prolonged duration Reuptake of endogenous NA or enzymatic degradation does not seem to be quantitatively important in affecting the transmitter in the thin walled portal vein when stimulated *in vitro* (Häggendal *et al* 1970) Therefore possible variations in these processes with different exogenous NA levels should not be of major importance for the present results However the presence of exogenous NA in the tissue may alter the amount of transmitter liberated per impulse due to neuronal uptake and subsequent release The influence of this mechanism cannot be assessed at present but the sequence of decreasing neurogenic potentiations observed with increasing exogenous NA concentrations (Fig 2) renders it unlikely that any important distortion of the present experiments would result from such an effect Although the mentioned reservations have a bearing on the shape of the transmitter concentration profile the present results support experimentally the conclusion from our recent theoretical analysis (Johansson *et al* 1972) that each transmitter concentration peak leads to a very high but shortlasting degree of α receptor occupation in the rat portal vein

In recent years the local transmitter concentrations attained during nerve activity have been calculated for different vascular neuroeffectors Such values are based on data for release elimination and distribution of the transmitter and on the functional distance between the nerve terminals and the effector cells In large elastic arteries the neuromuscular gap is very wide (Verity and Bevan 1968) and neurogenic activation of the deeper layers of the media is dependent on diffusion of the transmitter for considerable distances The calculated transmitter concentrations in elastic arteries (Bevan Chesher and Su 1969 Su and Bevan 1971) therefore are much lower than corresponding values arrived at for the perfused cat calf muscle preparation 6 10^{6} M (Folkow Häggendal and Lisander 1967) for the rat portal vein 4—8 10 M (Ljung 1970 Johansson *et al* 1972) and for the guinea pig uterine artery 4 10^{4} M (Bell and Vogt 1971) In these vessels the minimum neuromuscular distances are at least one order of magnitude less than in the elastic arteries and myogenic conduction from the innervated muscle cells seems to spread the neurogenic excitation through the media The variation between the transmitter concentration values in the latter studies is understandable in the light of the differences in the experimental approaches involved and the uncertainties of the basic assumption that are required for such calculations It seems conceivable that in blood vessels with propagating smooth muscle and with neuromuscular distances in the order of 1000 Å the important characteristics of the trans

mitter concentration profile resemble those described for the rat portal vein (Ljung 1970 Johansson *et al* 1972) The functional properties of the *α* adrenergic receptors apparently are uniform in different tissues (Furchgott 1967) and therefore the conclusions of the present study may be valid for the effective transmitter concentrations operating in many different blood vessels

Previous experiments based on the use of partial *α* receptor blockade (Ljung 1969) were designed to elucidate the effective transmitter concentrations in the rat portal vein In the face of our current understanding of the neuromuscular transmission in blood vessels however it appears that those results more accurately provide a comparison between the receptor activation obtained by nerve stimulation and exogenous NA respectively The results of that study are in good agreement with the present ones Bevan and Su (1971) have analyzed the mechanism underlying the widely accepted concept that *α* receptor blocking agents more effectively block responses to exogenous NA than to equipotent frequencies of vasomotor nerve activity Their results demonstrate that comparatively high local transmitter concentrations are important for the neurogenic responses also in a large elastic artery

In conclusion blood vessels within the vascular sections studied to date seem to be controlled by vasoconstrictor nerve influences operating with comparatively high transmitter concentrations In blood vessels with spontaneously active smooth muscle and neuromuscular distances around 1000 Å each nerve impulse seems to lead to very high or possibly maximal *α* receptor activation as evidenced by the present results on the rat portal vein

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Acta physiol scand 1972 85 297—304

From the Department of Zoophysiology University of Uppsala Sweden

The Metabolism of Liver Cholesterol in the Young Chick

By

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Abstract

SVANBERG O and A SVEDJELUND *The metabolism of liver cholesterol in the young chick* Acta physiol scand 1972 *85* 297—304

A mixture of ^{14}C-4 cholesterol and ^{14}C 26-cholesterol was injected into the invaginated yolks of newly hatched chicks which were then placed in metabolism chambers By tracing the label in expired air excreta (faeces + urine) and in their carcasses the main pathways for the metabolism of the liver cholesterol could be estimated quantitatively Over a period of 10 days about 26 % of the administered cholesterol was catabolized to bile acids about 19 % was eliminated from the body as neutral steroids in the excreta and at the end of the 10th day about 55 % was retained in the carcasses About 85 % of the bile acids synthesized was *recovered in the excreta As much as 45 % of the radioactivity derived from the* ^{14}C-4-cholesterol in the excreta was associated with the neutral steroids while 55 % was found in the bile acid fraction The rate of bile acid biosynthesis estimated from the ^{14}CO production decreased from the fifth day after hatching No estimation could be carried out during the days immediately following injection until isotopic equilibration had been reached The faecal excretion of neutral steroids increased rapidly up to the fifth day after which time it decreased The body appears to treat part of the pre formed cholesterol from the yolk as a surplus which has to be eliminated The specific activity of the cholesterol in the excreta collected on the 10th day was only about half of that of the intestinal content analyzed at the end of the same day

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The distribution after hatching of the huge amount of cholesterol esters accumulated in the liver of the chick embryo during the days immediately prior to hatching (Tsuji Brin and Williams 1955 Davison *et al* 1958) has recently been the subject of autoradiographic studies (Svanberg 1970 and 1971) Since no unexpected route of utilization of cholesterol was detected which could possibly be of any quantitative importance it was felt that a quantitative examination of the main metabolic pathways of cholesterol would be of great interest This might lead to a better understanding of the function of cholesterol in the developing chick and of its accumulation in the liver

In this investigation cholesterol labelled in the nucleus and in the side chain was injected into the yolks of newly hatched chicks The appearance of the radioactivity in the bile acids and in the neutral steroids of the excreta was recorded over a period of 10 days The expired ^{14}CO and the ^{14}C cholesterol retained in the carcasses was also measured

Experimental method and materials

Animals

In a first experiment 10 White Leghorn chicks were injected on hatching with a mixture of ^{14}C-4-cholesterol (46) and ^{14}C-26-cholesterol (54 %) emulsified according to Bergstrom and Wintersteiner (1941) The injections were made into the spare yolks about 2 mm cranial to the navel through the abdominal wall Each chick received a dose of 4 25 μc

A commercial standard food for young chicks was supplied Water was given *ad libitum*

The chicks were divided into 2 groups Each group was placed in a metabolism chamber making it possible to collect the CO expired and the excreta After 10 days the animals were killed using ether The yolks, if remaining were removed and stored together with the carcasses at −20 C prior to analysis

In a second experiment the size of the experimental groups was reduced to 3 This was considered necessary since the weight increase in the first groups was rather small In these chicks the alimentary canals were also opened and their contents collected for separate analysis

Metabolism chambers

The experimental apparatus was a modified version of that of Colvin Frahm and Morrison (1969) The metabolism chambers were constructed from methyl metacrylate with wire mesh floors The latter were fitted with removable drawers for the collection of excreta The food was taken by the chicks through an adjustable slit in the wall separating the food containers from the rest of the chamber Using this arrangement the contamination of the excreta with food was negligible This was found to be of importance in the extraction of the bile acids The total volume of the chambers was 12 l Air was drawn through the chambers by a water suction pump at a rate of 1 3 l/min To avoid condensation on the walls of the cage much of the humidity in the incoming air was removed by passing it over "porous CaCl The air leaving the chambers was bubbled through three sequential scrubber towers each containing about 150 ml 5 M NaOH to trap the CO To check the efficiency of the absorption a bottle containing a saturated solution of Ba(OH) was connected after the third trap The temperature in the cages was kept at approximately 37 C using heating bulbs

Every 24 h the chambers were opened to remove the excreta and fill the food containers At the same time the scrubber towers containing NaOH were exchanged

Determination of radioactivity

All radioactive samples were counted in a Nuclear Chicago Mark II liquid scintillation counter Scintillation systems were for steroids toluene containing 0 5 % PPO (w/v) and 0 03 dm-POPOP[b] (w/v) and for CO dioxane containing 10 naphtalene (w/v) 0 7 % PPO (w/v) and 0 03 dm POPOP (w/v)—in the future to be referred to as scintillation system 1 and 2 respectively

The counting efficiency for the ^{14}CO samples was determined by internal standardization (Colvin Frahm and Morrison 1969) and for the rest of the samples by the external standard channels ratio method

The trapping solutions in the scrubber towers were pooled and duplicate samples of 1 0 ml were transferred to scintillation vials The sodium carbonate was precipitated with 2 ml methanol and 10 ml of scintillation system 2 added Cab-O Sil was finally added to form a stable suspension after shaking The samples were kept in the dark at 4 C to remove chemoluminiscence

Extraction and fractionation

The daily collected excreta were dried at room temperature After grinding in a mortar they were extracted for 3 days with chloroform methanol 1 1 (v/v) in a Soxhlet continuous extraction apparatus The long time of extraction and the relatively high proportion of methanol was found to be necessary to completely remove the bile acids Samples of the extracts were taken for determination of the radioactivity The bile acids and the neutral steroids in the extract were fractionated in separation funnels according to Wilson (1964) and the radioactivity in each fraction was measured The amount of β sterols was determined according to Sperry and Webb (1950)

a PPO = 2 5-diphenyloxazole Packard Instrument Co)
b dm-POPOP = 1 4 bis-2 (4 methyl 5-phenyloxazolyl) benzene (Packard Instrument Co)
c Trade mark of Cabot Corp Boston Mass

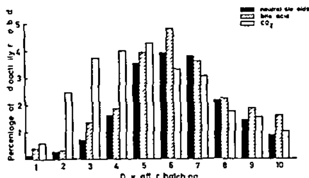


Fig 1 Radioactivity in the carbon dioxide expired and in the neutral and acid steroids of the excreta from chicks which on hatching had been injected in their invaginated yolks with a mixture of ^{14}C-4 cholesterol and ^{14}C 26 cholesterol The values are expressed as percentages of the radioactivity resorbed from the yolks at the end of the 10th day The values given are the means for 3 chicks

In order to evaluate the composition of the neutral steroid fraction samples were run on thin layer plates* with toluene ethyl acetate 9 1 (v/v) The substances were made visible with iodine vapour and the spots corresponding to cholesterol were scraped and eluted with chloroform methanol 3 1 (v/v) (Kritchevsky and Malhotra 19 0) After evaporation of the solvent in scintillation vials the steroids were dissolved in 10 ml of scintillation system 1 The radioactivity in the cholesterol spots was compared to that of equal samples spotted on the plates above the solvent front

Thin layer chromatography was also carried out on the bile acid fraction The radioactivity in each identified bile acid was determined as in the case of the neutral steroids The plates were developed with a solvent system consisting of the upper phase of mixed toluene glacial acetic acid water 50 50 10 (v/v) The spots were made visible by spraying with water (Waldi 1965)

The contents of the gut were extracted and analyzed in the same way as the excreta The carcasses and the yolks were homogenized separately lyophilized and extracted with chloroform methanol 2 1 (v/v) in a Soxhlet extraction apparatus The cholesterol content and the radioactivity of the extracts were measured as above

^{14}C-4 cholesterol (61 7 mCi/mmol) and ^{14}C 26-cholesterol (60 mCi/mmol) was purchased from the Radiochemical Centre Amersham England

All solvents were of analytical grade

Results

The weight increase of the chicks during the 10 days of the experiment was about 8 5 g/individual in the first experiment and 28 5 g/individual in the second experiment The amount of ^{14}C-cholesterol not resorbed from the yolks varied from 0 2 to 5 3 % of the injected radioactivity The highest values were obtained in the first experiment in which the chicks received too little food for optimal growth In spite of this all data registered from the four experimental groups coincided very well so that in the presentation of the results one of the groups of three will represent the complete data set if not stated otherwise

The radioactivity in the expired carbon dioxide which is considered to be a measure of the synthesis of bile acids from ^{14}C 26-cholesterol increased rapidly during the first few days and reached a maximum on the fifth day (Fig 1) The accumulated ^{14}C activity during the 10 days was 0 59 μCi/chick which is 25 9 % of the resorbed ^{14}C 26-cholesterol activity

* DC Fertigplatten Kieselgel (Merck AG Darmstadt Germany)

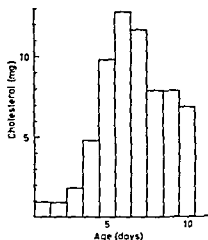


Fig. 2. Cholesterol in the excreta from chicks during the 10 days after hatching. The values given are the means for 3 chicks.

The ^{14}C content of the extracts from the excreta showed a variation pattern similar to that of the expired CO. The highest values were found one or two days later however (Fig. 1). After separation of the acid and neutral steroids the highest activity was found in the latter fraction. During the 10 days of the experiment 18.5 % of the resorbed ^{14}C activity (^{14}C-4-cholesterol and ^{14}C-26-cholesterol) was recovered in the neutral fraction. 22.1 % of the resorbed ^{14}C-4 cholesterol activity was found in the acid fraction. During the last few days of the experiment the activity of the bile acids gradually became higher compared to that of the neutral steroids.

The amount of digitonon precipitable steroids in the excreta also increased very rapidly from the first day to a maximum on the 5th day in the first experiment and

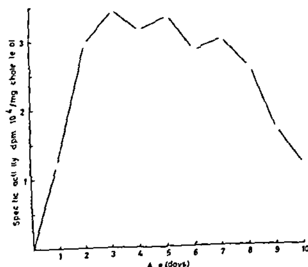


Fig. 3. Specific activity of cholesterol in the excreta from chicks which on hatching had been injected in their invaginated yolks with ^{14}C cholesterol. The values given are the means for 3 chicks.

TABLE I Recovery of ^{14}C activity from chicks injected on hatching with a mixture of ^{14}C-4 cholesterol (46 %) and ^{14}C-26-cholesterol (54) Excreta and carbon dioxide in the expired air were collected for 10 days (dpm × 10⁻ /chick) The values given are the means for 3 chicks

Excreta		CO	Gut		Carcass	Yolk	Total
Neutral steroids	Bile acids		Neutral steroids	Bile acids			
1741	*956*	*1314*	*159*	*98*	*4778*	*20*	*9067*
18 5 [a]	22 1 [b]	25 9	1 7	2 3 [c]	50 7 [a]	—	96 3

[a] percentage of ^{14}C-cholesterol resorbed from the yolk.
[b] ^{14}C-4-cholesterol resorbed from the yolk
[c] ^{14}C-26-cholesterol resorbed from the yolk.

on the 6th day in the second experiment (Fig 2) After this time there was a rapid decrease which was still continuing on the last day of the experiment

Thin layer chromatography revealed that 90—100 % of the radioactivity in the neutral steroid fraction migrated with cholesterol on the plates The specific activity of the cholesterol in the excreta decreased little from the third to the 8th day of the experiment (Fig 3)

The radioassay of the various bile acids isolated by thin layer chromatography revealed that 50—90 % of the radioactivity in the bile acid fraction from the excreta was associated with the spots on the chromatograms corresponding to chenodeoxycholic acid + deoxycholic acid These acids were not separated in the developing system used Less radioactivity was found in the spots with Rf values equal to that of cholic acid and lithocholic acid Slight ^{14}C activity was also detected in a faster running steroid which was not further characterized

In the second experiment the contents of the alimentary canal at the end of the experiment (end of the 10th day) was found to contain 5 9 mg cholesterol per chick This was nearly the same as that found in the excreta collected during the 10th day The specific activity however was twice that of the cholesterol in the excreta (Fig 3)

The recovery of ^{14}C in the carcasses on the 10th day varied between 42 and 51 % of the ^{14}C cholesterol resorbed from the yolk (Table I)

Discussion

^{14}C-cholesterol introduced into the yolks of newly hatched chicks very soon becomes homogeneously distributed in the yolk (Svanberg 1971) From here the labelled cholesterol is transported first into the liver where a marked accumulation occurs and after a few hours into most tissues of the body On the 9th day after hatching the radioactivity of most organs including the liver is very similar

Cholesterol is utilized in the animal organism as a precursor substance of bile acids and steroid hormones as well as a structural component in membranes In the catabolic processes the bile acid formation is quantitatively the most important

Part of the side chain is split off in both these metabolic events and the resulting propionic acid and isocaproic acid is further metabolized into carbon dioxide and water (Danielsson 1963 Chevallier 1967) Since the animal cell is not able to split the nucleus of the cholesterol molecule (Chaikoff *et al* 1952 Chevallier 1967) the ^{14}CO in this experiment will be derived from ^{14}C 26-cholesterol and constitutes a measure of the rate of bile acid synthesis as soon as isotopic equilibrium is reached in the liver The formation of steroid hormones from a quantitative view is insignificant (see Danielsson and Tchen 1968)

The labelled bile acids in the excreta were derived from ^{14}C 4 cholesterol Since about equal amounts of cholesterol labelled in the nucleus and in the side chain were injected the ^{14}C activity in the carbon dioxide produced should be the same as that of the bile acids in the excreta However the radioactivity in the expired air rose rapidly to a high value on the third day whilst that of the excreted bile acids was much lower during the first days This is probably due to the great reabsorption of bile acids in the gut by means of which these enter the enterohepatic circulation From the 7th day there was a fairly good resemblance between ^{14}CO and ^{14}C bile acids—an equilibrium is obviously reached between production and elimination of bile acids The fact that the ^{14}CO production decreased rapidly from the 5th day whilst the specific activity of the liver cholesterol was rather constant up to the 8th day judging from the specific activity of the neutral steroids in the excreta indicates that the rate of bile acid biosynthesis decreases from the 5th day A possible explanation of this is the negative feedback exerted by the bile acids returning via the portal vein to the liver (Beher 1962 Danielsson and Tchen 1968)

The most remarkable observation in this investigation is the very high excretion of cholesterol during the period when the cholesterol content of the liver is rapidly diminishing (Entenman Lorentz and Chaikoff 1940) The pre formed yolk cholesterol would appear to be temporarily deposited in the liver to be eliminated from the body unchanged after some time as a surplus without having any obvious function in the body In this connection it is of interest to note that laying hens eliminate cholesterol from the body via the eggs to about the same extent as via the faeces (Connor Osborne and Marion 1965 Andrews Wagstaff and Edwards 1968) and that hens with induced hypercholesterolemia lay eggs with a higher cholesterol content than normal hens (Harris and Wilcox 1963 Edwards and Jones 1964) The elevated cholesterol concentrations in the livers of newly hatched chicks is thus perhaps simply a reflection of a unique steroid excretion

No attempt has been made to distinguish between the cholesterol in the excreta of biliary origin and that of intestinal origin (secreta and sloughed cells) Neither have urine and faeces been separated The relative contribution of these routes to the steroids in the excreta varies among different animal species (Danielsson and Tchen 1968 Chevallier 1967) The fact that the specific activity of the cholesterol in the excreta during the 10th day was only about half of that of the intestinal content at the end of this day points to a pronounced dilution of the labelled biliary cholesterol with non labelled cholesterol The latter is probably synthesized in the

intestinal wall The intestine is next to the liver of major importance in synthesizing cholesterol (Lindsey and Wilson 1965 Danielsson and Tchen 1968)

In the adult fowl about 90 % of the radioactivity excreted after administration of ^{14}C 4 cholesterol is associated with the bile acid fraction (Nishida Ueno and Kummerow 1960 Sugano 1967) In the present experiment however this value was only about 54 % over the period of 10 days

From the fourth to the 8th day only about 50 % was found in the bile acids but during the last two days more was gradually recovered in this fraction It should be noted that both ^{14}C 4 cholesterol and ^{14}C 26 cholesterol are found in the neutral steroids whilst the labelled bile acids are only derived from the ^{14}C 4 cholesterol The former values must therefore be divided by two in order to be directly comparable with the neutral steroids The composition of the bile acid fraction was similar to that of 5 week-old chicks (Nishida Ueno and Kummerow 1960)

The small amounts of other steroids in the neutral steroid fraction indicates a minute microbial action on cholesterol in the gut This may be a result of raising the animals in the laboratory

About 50 % of the injected ^{14}C-cholesterol remained in the carcass on the 10th day The radioactivity is at this time evenly distributed in the body with the exception of the endocrine glands and the nervous system (Svanberg 1971) A relatively much higher retention was observed by Sugano (1967) in an experiment performed on adult chickens The specific activity of the cholesterol in the excreta decreased rather rapidly from the 8th day This coincides with the onset of the hepatic cholesterol biosynthesis reported by Goodridge (1968) and might be explained by this fact However the direct secretion of cholesterol synthesized by the intestinal wall may also contribute significantly to this decrease (Chevallier 1967) In fact the time dependence and a relative importance of the latter process is not known in chicks

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Secretion from the Dog's Parotid at Different Time Intervals after Denervation

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Abstract

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Previous observations suggest that cholinergic secretory fibres reach the dog s parotid gland not only via the auriculo-temporal nerve but also along the internal maxillary artery 3—5 days after section of both these sets of nerves citric acid applied to the oral mucosa was found to evoke slow secretion only or none at all It has been reported however that the secretory response elicited reflexly after auriculo-temporal nerve section gradually increases during the days following the operation The auriculo-temporal nerve was divided unilaterally in one group of dogs and in another group this operation was combined with section of the nerves on the internal maxillary artery During the following five weeks the dogs were examined repeatedly Each time the secretory innervation was studied in two ways by pouring citric acid into the mouth and by injecting eserine through the salivary duct into the gland. In addition the secretory responses to methacholine were estimated The results indicate that postoperatively there was first a period in which there was some increased release of acetylcholine in the gland (a degeneration phenomenon) supersensitivity then developed in the secretory cells In spite of these complications both methods to study the secretory innervation could be used after the first week and onwards to demonstrate the difference in the effectiveness between the two denervation procedures

Recent experiments suggest that some cholinergic secretory fibres to the parotid gland of the dog run outside the auriculo-temporal nerve on the internal maxillary artery (Holmberg 1971) After section of the auriculo temporal nerve marked secretion could still be induced reflexly but the responses were much diminished when the fibres on the artery were cut as well in fact in about half the experiments no secretion at all was obtained In the others however there was a small but definite secretion suggesting that some secretory nerves remained The effectiveness of the attempted denervation was in these experiments estimated after 3—5 days when the animals had recovered well from the operation There are reasons to suspect however that the secretory response to reflex stimulation might vary considerably at different time intervals after denervation Thus Baxter (1928) according to Babkin found that During the first few days after transsection of the left auriculo temporal nerve the secretion of the ipsilateral parotid gland in response to food was very scanty but gradually the volume of the secretion increased to about 20 % of that

of the normal gland on the 10th day. Several events in the gland consequent on the denervation might cause such an increased response to reflex stimulation. Supersensitivity of salivary gland cells is a wellknown effect of denervation but its time of onset in the parotid gland of the dog is unknown and likewise whether it would affect glandular cells still supplied with some remaining secretory fibres. Furthermore, a few days after denervation there may be a period during which transmitter is released from the degenerating nerve in amounts sufficient to evoke secretion (see Emmelin 1967). It seems likely that such a degeneration secretion occurs in the parotid gland of the dog since it has been found in all other salivary glands so far investigated including the submaxillary gland of the dog. In addition collateral sprouting may take place from the persisting secretory nerves at a fairly early stage and later on conventional regeneration.

In view of these possible complicating factors it was decided to make repeated observations on each dog at different times after section of the auriculo-temporal nerve or this nerve and the new secretory fibres on the artery. Each time the size of the reflexly induced secretion was estimated and also the responses of the glands to standard doses of methacholine and eserine. By injecting methacholine the responsiveness of the glandular cells could be determined. Eserine applied in such a way as to restrict its action to the salivary gland is assumed to cause secretion by preserving acetylcholine leaking from the cholinergic nerves in the gland; a secretory response to eserine is thus taken as evidence to show that such nerves are present in the gland (Emmelin and Strömblad 1958, Emmelin and Perec 1968).

Material and Methods

Eighteen mongrel dogs were used. The functional state of their parotid glands was tested repeatedly according to the following procedure. After an i.v. injection of a barbiturate (sodium thiopentone 30 mg/kg) polyethylene tubes were introduced into the oral orifices of the two parotid ducts. The maximal secretory rate provoked by pouring citric acid on the tongue of the animal was estimated as described earlier (Holmberg 1971). Then the level of the anaesthesia was deepened with a small additional dose of the barbiturate. The recording system was changed from the [illegible] to measure the flow rates to electromagnetic pens writing on a smoked drum. Hexamethonium bromide 10 mg/kg was given to abolish reflex stimulation of salivation during the rest of the experiment. First the secretory response to methacholine 1 µg/kg injected into the left ventricle of the heart was recorded, then eserine sulphate 30 µg in 0.3 ml saline was injected through the parotid ducts into the glands according to the method described by Emmelin, Muren and Strömblad (1954). In innervated glands secretion started after one to three min. It was recorded for a period of 15 min.

When these tests had been made on different days separated by one day or more, at least one more day of rest was allowed. Then the right auriculo-temporal nerve was exposed in ether anaesthesia according to Burgen ([illegible]) and divided at the mandibular joint in 9 animals. In the other 9 dogs section of the nerve at the same level was combined with division of the secretory fibres on the right internal maxillary artery as described earlier (Holmberg 1971). The functional state of the glands was tested on the 2nd, 4th and 6th post-operative day and then with weekly intervals. The normally innervated left gland served as a control of the condition of the animal. The postoperative secretory responses evoked by the three methods used were calculated as percentages of the two preoperative estimations on the same gland. The percentage [illegible] between the two groups of animals at each interval postoperatively [illegible] with Student's t-test for samples of unequal size using [illegible] disturbed some of the experiments. The symptoms were [illegible] breathing and the occurrence of dark blood in the [illegible]. The results of these

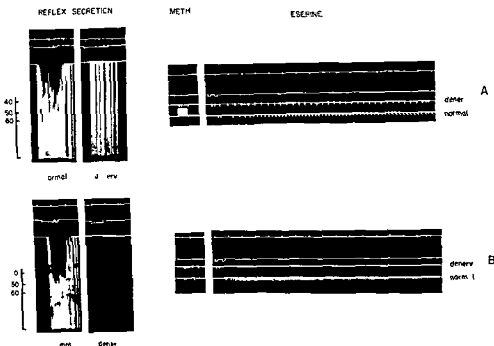


Fig 1 Secretory responses in 2 dogs A and B 4 days after unilateral section of the auriculotemporal nerve (A) and this nerve and the nerves on the internal maxillary artery (B) Each part of the tracings shows from above minutes signal and salivary flow To the left secretion was induced reflexly with citric acid at first signal followed by water at the second one The flows of saliva from the normal and the operated glands were recorded simultaneously with ordinate writers (Calibrations in drops/min at extreme left) To the right secretion was elicited by methacholine 1 μg/kg intracardially and by eserine 30 μg in 0 3 ml injected through the salivary duct Drops of saliva from the denervated and below it from the normal gland were recorded using and electromagnetic pen

test occasions obtained after the complication were excluded from the series but if the animal survived a new test on it was later made according to the time table In 2 tests on the 2nd day reflex stimulation provoked a longlasting secretion in the denervated gland which did not cease after hexamethonium In these tests the response to methacholine could not be separated from th s provoked secret on and was therefore excluded from the material

Results

In Fig 1 are shown records obtained on the 4th postoperative day in two different animals In dog A the auriculo temporal nerve had been divided on one side where as in dog B this operation had been combined with section of the nervous strands on the internal maxillary artery Secretion induced with reflex stimulation and eserine administration was found to be reduced in the partially denervated gland of dog A Neither of these stimuli however could provoke any secretory response at all from the more extensively denervated parotid of dog B in spite of the increased excitability to injected methacholine found in the secretory cells of this gland compared with the contralateral one Judging from the two criteria used it would

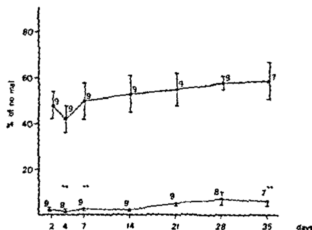


Fig 2 The secretory responses of the right parotid to reflex stimulation obtained on different days postoperatively in 9 dogs where the right auriculo-temporal nerve was divided (dots) and in 9 animals where section of this nerve was combined with division of nerve strands on the int max artery (triangles) The responses are expressed as % of the mean of two preoperative responses obtained on the same gland Figures near the points Number of glands included Brackets ±S E of the mean *** p < 0 001

then appear that in these two examples the more extensively denervated gland of dog B was totally deprived of secretory nerves and the less extensively operated one of dog A was partially denervated Although the results obtained were not always as clearcut as in these examples it is evident from Fig 2 that reflexly induced secretory rates were significantly lower at all intervals postoperatively in the glands where both the auriculo temporal nerve and the fibres on the internal maxillary artery had been divided than in those where only the former nerve had been cut From this finding it can be concluded that the combined section of both nerves produced a more effective denervation of the gland than section of the auriculo temporal nerve only This conclusion gains further support from the results summarized in Fig 3 that the eserine induced secretory rates were significantly lower from the 7th postoperative day in the more extensively denervated glands The high secretory responses to eserine on day 2 and 7 are probably not mainly caused by the normal leakage of acetylcholine from intact nerve fibres but are more likely to be the result of an increased leakage of acetylcholine from the degenerating nerve terminals In other

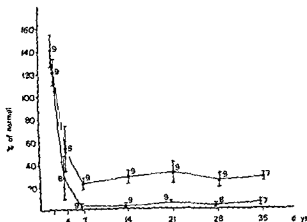


Fig 3 The secretory responses of the right parotid to local eserinization obtained at different days postoperatively in 9 dogs where the right auriculo temporal nerve was divided (dots) and in 9 animals where section of this nerve was combined with division of nerve strands on the int max artery (triangles) The responses are expressed as % of the mean of two preoperative responses obtained on the same gland Figures near the points number of glands included Brackets ±S E of the mean ** p < 0 01 * * p < 0 001

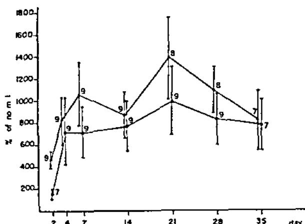


Fig 4 The secretory responses of the right parotid to the intra cardial injection of methacholine 1 μg/kg obtained at different days postoperatively in 9 dogs where the right auriculo-temporal nerve was divided (dots) and in 9 animals where section of this nerve was combined with division of nerve strands on the int.max artery (triangles) The responses are expressed as % of the mean of two preoperative responses obtained on the same gland Figures near the points number of glands included Brackets ±S E of the mean There was not any significant difference between the two groups at any time interval post operatively

salivary glands such an increased leakage of acetylcholine manifests itself as degeneration secretion even when eserine has not been given (see Emmelin 1967) but this phenomenon was not observed in the present experiments

It is also evident from Fig 2 and Fig 3 that although the secretory responses to reflex stimulation and to eserine were very slight after the more extensive operation they were not in general totally abolished at all intervals postoperatively This indicates that the glands were not completely denervated The mean secretory responses as well as the number of glands responding increased slightly with the course of time reflex stimulation produced secretion in 2 of the gland on the 2nd day compared with 5 glands on the 35th day Correspondingly only 2 of the glands responded to eserine on the 7th day when the mean responses to this drug were smallest whereas secretion could be obtained in 5 glands on the 35th day All the glands produced some saliva in response to eserine or reflex stimulation at one time or other during the observation period so apparently none of them was completely denervated

The very small increments found with the course of time in the secretory responses to eserine and to reflex stimulation were probably only to a very minor extent caused by early nerve regeneration They were more likely to be due to subthreshold degeneration secretion first and to supersensitivity of denervation later as was the marked enhancement of the secretory responses to methacholine summarized in Fig 4

Discussion

The present investigation supports the previous finding (Holmberg 1971) that the parotid gland of the dog is more effectively denervated when not only the auriculo temporal nerve but also the secretory fibres on the internal maxillary artery are divided because the secretory responses to reflex stimulation and local eserinization were found to be smaller after the more extensive operation Reflexly induced secre-

tory rate was significantly smaller from the 2nd postoperative day, that produced by eserine from the 7th day postoperatively On the 2nd postoperative day eserine provoked a secretory rate which was higher than that of the normal glands Most likely the degenerating nerves released increased amounts of acetylcholine at this time although the quantities were below the secretory threshold in the absence of eserine In other salivary glands degeneration secretion occurs even in the un eserinized gland at about this time after division of the secretory nerves (Emmelin 1967), but this was not observed in the present experiments Eserine induced secre tory rate can probably not be considered as an index of the number of normal not degeneraing nerves in the gland until the 7th postoperative day when the smallest responses were recorded

The pronounced increases in the responses to injected methacholine seen re markably early after the operation can probably be explained in a similar way they were not due to supersensitivity of the effector cells at this early stage but a conse quence of the fact that increased amounts of acetylcholine were liberated adding their secretory effect to that of the methacholine

The observations with eserine and methacholine thus suggest that in the parotid gland of the dog as in other salivary glands there is a period of increased release of acetylcholine while nerves are degenerating The fact that an overt degeneration secretion was not obtained may be due to a slight atropine effect of the barbiturate (Emmelin 1952) that was used in the present investigation

Apparently there are some nerves to the parotid outside those already discovered because small but definite secretory responses to reflex stimulation and eserine ad ministration could be recorded from all the extensively denervated glands These responses appeared too early after the operation to be mediated by regenerated nerves They were more likely produced via persisting nerves It was not possible to ascertain to what extent the secretory rates induced by reflex stimulation and eserine administration were increased by the supersensitivity developed because during no period of time could a basal level be recorded for these responses which with certain ty was unaffected by the additive effect of an increased leakage of acetylcholine or by supersensitivity of the glandular cells Since no significant difference in super sensitivity to methacholine was found between the more and the less extensively operated glands however supersensitivity does not appear to be a factor which greatly disturbed the comparisons of the reflexly and eserine induced secretory rates made between the glands denervated according to the two methods Nerve regenera tion seemed to occur only to a very slight degree during the observation period if it occurred at all

The experiments thus show that although there are signs of early increase in the acetylcholine release and later on of supersensitivity these and other possible changes in the neuroglandular region cannot obscure the fact that the more extensive oper ation caused by far the more effective denervation of the gland with the method of reflex stimulation this could be demonstrated from the second day and with the eserine method after the first week and in both cases for several weeks onwards

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Role of Cyclic AMP and Ca++ in the Metabolic and Relaxing Effects of Catecholamines in Intestinal Smooth Muscle

By

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Abstract

ANDERSSON R *Role of cyclic AMP and Ca in the metabolic and relaxing effects of catecholamines in intestinal smooth muscle* Acta physiol scand 1972 85 312—322

In rabbit colon muscle there was a quantitative correlation and a co-ordination in time between relaxation and an increase in cyclic AMP on stimulation of adrenergic β receptors The cyclic AMP content had increased before the muscle had started to relax There was an increase in the phosphorylase *a* activity and reduction of the ATP content The adrenergic β receptor blocking agent sotalol inhibited the relaxation and the increase in cyclic AMP content Reduction of the Ca content of the muscle decreased the cyclic AMP content and the phosphorylase *a* activity These effects were restored on addition of Ca ions In a Ca poor muscle adrenergic β receptor stimulation still produced an increase in cyclic AMP content and phosphorylase *a* activity but there was an increase in the ATP content instead The reduction of cyclic AMP which followed an adrenergic α receptor stimulation was eliminated in the Ca poor muscle The existence of a Ca accumulating ATP utilizing mechanism stimulated by cyclic AMP might explain the relation between relaxation cyclic AMP and Ca and adrenergic β receptor stimulation

Andersson and Mohme Lundholm (1969 a 1970) demonstrated in rabbit intestinal smooth muscle that the relaxation mediated by adrenergic β receptors was associated with and probably dependent on an increased content of cyclic AMP in the muscle The relaxation mediated by adrenergic α receptors was associated with a reduced cyclic AMP content The two types of relaxation also differed in many other respects In Table II the differences observed between the two kinds of relaxation are summarized the observations made in this study are also included Briefly the metabolic effects which preceded the adrenergic β receptor mediated relaxation comprised an increased cyclic AMP content an activation of phosphorylase *a* an increase of some carbohydrate metabolites and a decrease of the ATP and CrP contents

The relaxation mediated by adrenergic α receptors which had a shorter latency time, was initially not associated with any metabolic effects but was followed by reductions of the cyclic AMP content the phosporylase *a* activity and some carbohydrate metabolites and an increase of the ATP content

There is evidence that contraction and relaxation of skeletal and cardiac muscles are dependent on changes in the intracellular concentration of free Ca^{++} in the muscles (reviews Sandow 1965 Ebashi and Endo 1968) In smooth muscle there is increasing evidence too that Ca has the same function (Edman and Schild 1963 Daniel and Irwin 1965 Hurwitz Joiner and von Hagen 1967 Rüegg 1971)

It has been suggested (Schild 1967 Kuriyama 1970) that the relaxing action of the catecholamines in smooth muscle is also exerted via an effect on the Ca^{++} metabolism The sarcoplasmic or microsomal fraction from skeletal or cardiac muscle bind or accumulates Ca under utilization of ATP (Ebashi and Endo 1968) This Ca^{++} accumulation was in cardiac muscle stimulated by adrenergic β receptor stimulating agents and cyclic AMP (Entman Levey and Epstein 1969 Shinebourne *et al* 1969) It was considered that the ATP reduction produced by isoprenaline (Table II) or cyclic AMP on relaxation of smooth muscle might depend on stimulation of a Ca accumulating or binding mechanism In the following the relation between cyclic AMP and Ca has been investigated in intestinal smooth muscle Further evidence that the β receptor mediated relaxation is dependent on an increased formation of cyclic AMP are also presented A preliminary report has been presented previously (Andersson and Mohme Lundholm 1969 b)

Methods

The methods were generally similar to those used in earlier works (Andersson and Mohme Lundholm 1969 1970) The experiments were performed on rabbit colon muscle freed from mucosa and suspended in Krebs Henseleit bicarbonate buffer solution at 37 C gassed with 95 O_2 + 5 % CO_2 The muscle specimens prepared were 0 5—0 8 mm thick 12 mm wide 20 mm long and weighing 0 13—0 18 g They were attached to plastic holders as described by Lundholm and Mohme Lundholm (1966) and the isometric contraction was recorded by an FT 03 force transducer on a Grass polygraph The preparations were suspended for 60 min in the buffer before the drugs were added When the Ca content of the preparations was reduced they were suspended in a Ca^{++} free buffer to which was added 2×10^{-4} M of EDTA the buffer was changed several times After 60 min the spontaneous tension was abolished and the muscle did not respond to contracting agents (Fig 4) The preparation was then incubated in Ca^{++} free Krebs Henseleits buffer but without EDTA Normal concentration of Mg (1 ? mM) was present

To specifically stimulate adrenergic α or β receptors l phenylephrine or l isoprenaline in submaximal concentrations were used Earlier studies had shown that too high concentrations of phenylephrine or isoprenaline stimulated both α and β receptors With phenylephrine in a concentration of $\leqslant 1 \times 10^{-6}$ g/ml the relaxing action was totally blocked by the α receptor blocking agent dibenamine (5×10^{-6} g/ml) and the corresponding action of isoprenaline ($\leqslant 5 \times 10$ g/ml) was completely blocked by the β receptor blocking agent sotalol indicating that the drugs relaxed the muscle by specifically stimulating adrenergic α or β receptors (Andersson and Mohme Lundholm 1970)

After the drugs had been added and when the concentrations of metabolites was to be determined the muscle was frozen after different lengths of time at −80 C in frigen 1? containing solid CO_2 The phosphorylase activity was then determined according to Bueding *et al* 196? and the hexose phosphates lactate ATP and CrP were analyzed by enzymatic methods as described by Andersson and Mohme Lundholm (1970) Cyclic AMP was analyzed according to Kakiuchi and Rall (1968) The samples for calcium analysis were prepared by digestion with ultrapure HNO_3 and Ca was determined by means of a Unicam Atom spectrophotometer (Parker 1964)

The following drugs were used l phenylephrine chloride (Neo-Synephrine® Wintrop) l isoprenaline bitartrate (Cilag Chemi) ACTH (Isactid® Ferring) a crystalline chromatographic purified preparation from hog hypophysis sotalol (dl 4 (? isopropylamino-l hydroxyethyl) methane sulfonanilide HCl (M J 199? Mead Johnson) and dibenamine hydrochloride

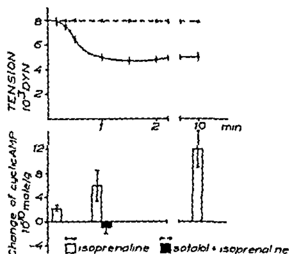


Fig 1 Time response relationship of the action of l isoprenaline (5×10^{-7} g/ml) on tension and cyclic AMP content before and after pretreatment with sotalol (1.2×10^{-5} g/ml) The control value of cyclic AMP = $8.2 \pm 1.8 \times 10^{-10}$ mol/g Mean±S E (n = 6—8) The statistical significance in paired test from the same colon muscle is denoted by * = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$

Results

A quantitative correlation and co ordination in time between relaxation and change in cyclic AMP content in rabbit colon

In rabbit intestinal smooth muscle isoprenaline (5×10^{-7} g/ml) had significantly increased the cyclic AMP content 10 s after its addition and before the muscle had started to relax (Fig 1) The increase in cyclic AMP was more marked at 60 s and persisted for a further 10 min while the muscle was still relaxed Pretreatment of the muscle with the adrenergic β receptor blocking agent sotalol (1.2×10^{-5} g/ml) inhibited the relaxation and the increase in cyclic AMP sotalol had no significant effect of its own on the cyclic AMP content (Fig 1)

There was a correlation between the increase in cyclic AMP and the degree of relaxation on addition of different doses of isoprenaline when after 60 s the relaxation had reached its maximal value (Fig 2)

The activation of phosphorylase *a* and reduction of the ATP and CrP contents of the muscle showed also a quantitative relationship to the relaxation (Fig 2)

5 s after stimulation of adrenergic α receptors by phenylephrine there was no change in cyclic AMP content ($-0.3 \pm 0.5 \times 10^{-10}$ mol/g) from the control value ($1.5 \pm 0.3 \times 10^{-9}$ mol/g) After a latency of 60 s there was a small but significant decrease of the cyclic AMP content At the same time there was a decrease of the phosphorylase *a* activity and an increase of the ATP content (Fig 3) The decrease of cyclic AMP was inhibited by dibenamine (5×10^{-6} g/ml) (Fig 3)

Metabolic effects of reduction of the Ca content in the intestinal muscle

After incubation of the muscle preparation for 60 min in a Ca free Krebs Henseleit bicarbonate buffer solution with 2×10^{-4} M EDTA its Ca content decreased (Fig 4) from $5.9 \pm 0.5 \times 10^{-3}$ mol/kg to $1.3 \pm 0.2 \times 10^{-3}$ mol/kg after 60 min At the same time (after 60 min) the contracting action of carbacholine was blocked (Fig 4) It

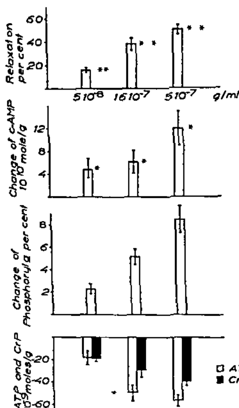


Fig 2

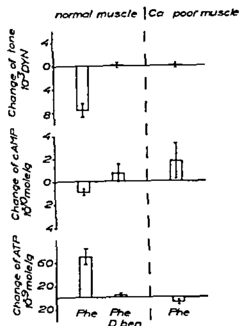


Fig 3

Fig 2 Dose response relationship of the action of 1 isoprenaline ($5\times10^{-8}-5\times10^{-7}$ g/ml) on mechanical and metabolic events in rabbit colonic muscle Relaxation = tension after isoprenal ne in per cent of initial tension Changes of cyclic AMP phosphorylase *a* ATP and creatinephosphate (CrP) from control value 60 sec after addition of isoprenaline Mean ± S E (n = 6—8) Statistical significance as in Fig 1

Fig 3 Influence of 1 phenylephrine (1×10^{-6} g/ml) alone or in combination with dibenamine (5×10^{-6} g/ml) on tension cyclic AMP content and ATP content of normal and Ca^{++} poor rabbit colonic muscle 60 s after addition of the drug Mean±S E (n = 5—8) Statistical significance as in Fig 1

is thus evident that the effect was related to a reduction of the Ca^{++} content of the muscle but that the muscle was in no way Ca free

The reduction of the Ca^{++} content of the muscles was associated after 60 min with a small but significant decrea e of the cyclic AMP content (Table I) and a reduction of the phosporylase *a* activity and ATP content When Ca^{++} in a final concentration of 3.0×10^{-3} M was re added to the Ca^{++} poor preparation the mu cle contracted initially but relaxed after about 5 min After the re addition of Ca the cyclic AMP content and the phosphorylase *a* activity had again increased after 60 s and returned to their initial levels but the ATP content was still depressed in relation to the normal muscle This latter effect was probably a result of the muscle contraction —

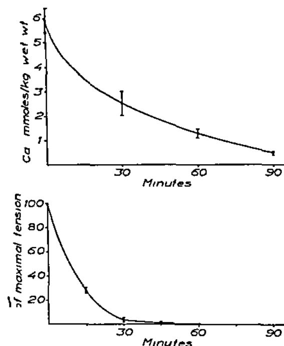


Fig 4

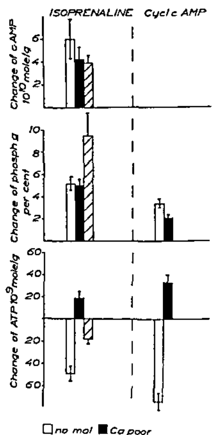


Fig 5

Fig 4 Upper curve Loss of total Ca from rabbit colonic muscle incubated in Ca free Krebs solution containing 0 2 mM EDTA Points are averages of 8 determinations Vertical bars indicate the S E Lower curve shows the effect of Ca depletion on contraction by carbacholine ($1\ 7\times10^{-7}$ g/ml) Each point represents the mean ± S E of 8 expts

Fig 5 Influence of l isoprenaline (5×10^{-7} g/ml) and cyclic AMP ($1\ 1\times10^{-6}$ mol/ml) on metabolic events in normal Ca poor and Ca poor Ba contracted colon muscle Changes of cyclic AMP phoshorylase *a* and ATP from control values 60 s after addition of the drugs Mean ± S E (n = 6—8) The level of significance is $p < 0\ 01$ for the average of the differences between treated and control muscles

which in smooth muscle is associated with a reduced ATP content (Bevız *et al* 1965)

Addition of Ba^{++} (4×10^{-3} M) to the Ca poor muscle contracted the muscle and produced a marked increase in cyclic AMP phosphorylase *a* activity and content of hexosephosphates (Table I) Isoprenaline relaxed the Ca poor Ba contracted muscle and reduced the ATP content (Fig 5) Ba can thus substitute for Ca both in the metabolic and mechanical effects in the β receptor mediated relaxation The Ba^{++} contracted Ca^{++} poor muscle was not relaxed by adrenergic α receptor stimulation

TABLE I Changes in phosphorylase *a* activity (per cent of total phosphorylase activity) cyclic AMP glucose 1 phosphate and ATP contents (nmol/g wet weight) of rabbit colonic muscle occurring after removal of Ca^{++} from the muscle and 60 s resp 10 min after addition of 3 mM Ca or 4 mM Ba^{++} to a Ca poor muscle Δ = change from control muscle Δ_s = change from the Ca^{++} poor muscle in paired muscles Significance of the effect is denoted by * = P < 0 05 ** = P < 0 01 n = number of tests

		n	cyclic AMP	Phosphorylase *a*	G 1 P	ATP
	control muscle	6	2 60±0 29	5 53±0 81	28±3	160±14
Δ	Ca poor muscle	6	−0 52±0 11 *	−3 53±0 50*	−8±3*	−86±15**
Δ_s	Ca^{++} added to Ca poor muscle	6	+0 36±0 09**	+4 47±0 95**	+5±2	+10±5
	control muscle	8	1 36±0 13	6 27±0 75	46±4	
Δ	Ba^{++} added to Ca poor muscle	8	+0 33±0 10*	+8 49±3 12*	+15±3 *	

Metabolic effects of adrenergic α and β receptor stimulation in the Ca^{++} poor muscle

In the Ca^{++} poor muscle preparation the cyclic AMP increasing action of isoprenaline was not changed (Fig 5) The phosphorylase *a* activating action was still present but the ATP reducing action of isoprenaline was blocked (Fig 5) ACTH relaxed the rabbit colon through stimulation of adrenergic β receptors (Andersson *et al* 1971) The cyclic AMP increasing action of ACTH was not changed in the Ca^{++} poor preparation After 90 sec incubation with ACTH there was a significant increase of the cyclic AMP content by $3\,3 \pm 0\,9 \times 10^{-10}$ mol/g from a basal value of $4\,9 \pm 1\,4 \times 10^{-10}$ mol/g (n = 5 p < 0 02) The corresponding increase in normal muscle was $2\,0 \pm 0\,46 \times 10^{-10}$ mol/g (n = 6 p < 0 01)

In further studies it was investigated how the metabolic actions of cyclic AMP itself were influenced in the Ca^{++} poor preparation Its phosporylase *a* activating action was not changed but its reduction of the ATP content was blocked and changed to an increasing action just as that of isoprenaline in the Ca^{++} poor muscle (Fig 5) The increase in ATP may be dependent on a selective stimulation of the phosphorylase *a* activity and the carbohydrate metabolism by isoprenaline and cyclic AMP

The reduction of the cyclic AMP content and phosphorylase *a* activity which followed an adrenergic α receptor stimulation by phenylephrine were eliminated in the Ca^{++} poor preparation (Fig 3)

Discussion

There was a quantitative and time dependent relationship between the increase of cyclic AMP and adrenergic β receptor mediated relaxation An adrenergic β receptor blocking agent blocked both the relaxation and the increase of cyclic AMP The simultaneous changes of the phosphorylase *a* activity and increase in hexosephos

TABLE II Differences in relaxation mediated in rabbit colon smooth muscle by adrenergic α and β receptors

Parameter	Adrenergic α receptor	Adrenergic β receptor
Drugs		
specific agonist	phenylephrine $< 1 \times 10^{-6}$ g/ml	isoprenaline $< 5 \times 10^{-7}$ g/ml
blocking agent	dibenamine 5×10 g/ml	sotalol (MJ 1999) 1.2×10^{-6} g/ml
theophylline 5×10^{-6} g/ml	relax unchanged	relax potentiated
deoxycorticosterone 1×10^{-6} M	relax blocked	relax unchanged
influence of prestimulation of adrenergic receptors on carbacholine contraction	unchanged	potentiated
Character of relaxation		
onset of relaxation	rapid > 5 sec	slow > 20 sec
degree of relaxation	almost complete	to 40–50
Experimental condition		
cold stored muscle	relax blocked	relax reduced
K^+-depolarized muscle	relax blocked	relax reduced
carbohydrate poor muscle	relax reduced	relax blocked
replacement of Ca^{++} by Ba^{++}	relax blocked	relax reduced
Metabolic events		
onset of events	after the relax	preceding relax
cyclic AMP content	reduced	increased
ATP and CrP content	increased	reduced (initially)
phosphorylase *a* activity	decreased	increased
hexose phosphate content	decreased	increased
lactate content	decreased	increased
Metabolic events in Ca poor muscle		
cyclic AMP content	unchanged	increased
ATP and CrP content	unchanged	increased
phosphorylase *a* activity	unchanged	increased

phates and lactate demonstrated that the increase in cyclic AMP content in smooth muscle was of an order that stimulated metabolic processes

Dobbs and Robison (1968) have demonstrated a relationship between the potency of different catecholamines to relax uterine smooth muscle and their cyclic AMP increasing action ACTH which relaxed intestinal smooth muscle by stimulation of adrenergic β receptors also increased the cyclic AMP content (Andersson *et al* 1971) Cyclic AMP itself relaxed intestinal smooth muscle and produced the same metabolic actions as an adrenergic β receptor stimulation (Andersson and Mohme Lundholm 1970) Adrenergic α receptor stimulation which relaxed intestinal smooth muscle by another mechanism (Table II) did not increase the cyclic AMP These findings indicate a relationship between adrenergic β receptor mediated relaxation and an increased cyclic AMP content The question of whether this relationship is a case of cause and effect or of parellelism cannot be finally answered until it is known how cyclic AMP produces a relaxing action in smooth muscle As contraction

and relaxation in skeletal and cardiac muscle are related to the concentration of free Ca in the sarcoplasm the question of the relationship between cyclic AMP and Ca^{++} in smooth muscle is of interest

An interesting observation regarding the relationship between the adenyl cyclase cyclic AMP system and Ca^{++} was made with the demonstration by Ozawa and Ebashi (1967) that the activation of phosphorylase *b* kinase by cyclic AMP needed small amounts (2×10^{-6} M) of Ca^{++} Rasmussen and Tenenhouse (1968) suggested on the basis of this and other observations that in a more general way cyclic AMP needed Ca^{++} to produce its effect During recent years it has become evident however that the relationship between cyclic AMP and Ca^{++} is more complicated than originally outlined (Rasmussen 1970) and that Ca^{++} influences different reactions (adenyl cyclase phosphodiesterase phosphorylase *b* kinase) in processes involving cyclic AMP

Adenyl cyclase Bar and Hechter (1969) demonstrated that the activation of adipose tissue adenyl cyclase by ACTH was Ca^{++} dependent whereas the effect of catecholamines was not The binding of ACTH to the receptor and activation of adenyl cyclase from the adrenal cortex were also Ca dependent but Ca^{++} in a higher concentration dissociated the agonist receptor complex and blocked the activation of adenyl cyclase (Lefkowitz Roth and Pastan 1970) Vigdahl Marquis and Tavormina (1969) reported that Ca reduced the stimulating action of prostaglandins on the cyclic AMP content of platelets

In this study neither the cyclic AMP increasing action of isoprenaline nor that of ACTH was significantly changed in the Ca^{++} poor preparation The absence of ATP reduction observed in the Ca^{++} poor preparation treated with isoprenaline can therefore not be explained on the basis of a reduced cyclic AMP formation As the muscles still contained a total of about 1.3×10^{-3} mol/kg of Ca it is evident that the results do not allow any definite conclusions as to whether a very low concentration of Ca^{++} is needed for the β receptor stimulation of adenyl cyclase or not An early observation that Ca in a high concentration blocked both the relaxing and metabolic effects of adrenaline in tracheal smooth muscle (Mohme Lundholm 1956) may indicate that a high concentration of Ca^{++} is able to inhibit stimulation of adrenergic β receptor function in smooth muscle

In the Ca^{++} poor preparation the cyclic AMP was reduced in comparison to normal muscle but increased again on readdition of Ca (Table I) effects which may be explained by the assumption that Ca^{++} influenced the adenyl cyclase activity These results may however not be dependent solely upon changes of the adenyl cyclase activity for the following reasons

The cyclic AMP reducing effect of phenylephrine was Ca^{++} dependent (Fig 3) The effect of phenylephrine was only evident after some delay when the relaxing action already was maximal and was probably therefore not caused by the relaxation but a sequence to it In a study reported in a forthcoming paper (Andersson 1972) it was found that phenylephrine activated the cyclic AMP hydrolyzing enzyme phosphodiesterase and that this activation was Ca dependent In intestinal smooth

muscle the main effect of phenylephrine might therefore be to increase the enzymatic hydrolysis of cyclic AMP Ca itself was able in low concentrations (1×10^{-7}–1×10 M) partially to inhibit the phosphodiesterase activity Some contracting drugs such as carbacholine and K ions increased after some delay the cyclic AMP content of the muscle The effect was combined with an inhibition of the phosphodiesterase activity it was absent in the Ca poor preparation Ca itself probably influenced the cyclic AMP content of the muscle at least partly via the phosphodiesterase activity

Ca^{++} and phosphorylase activity Cyclic AMP and Ca activate phosphorylase *a* by different mechanisms but synergetically by stimulating the phosphorylase *b* kinase activity Cyclic AMP increases the amount of active phosphorylase *b* kinase, whereas *low concentrations of Ca (2×10^{-6} M) are necessary for the action of active* phosphorylase *b* kinase (Ozawa and Ebashi 1967) Adrenaline thus activates skeletal phosphorylase *a* via cyclic AMP whereas the activation associated with muscular contraction is not combined with cyclic AMP changes (Posner *et al* 1964) but is probably dependent on an increased Ca^{++} concentration (Walsh *et al* 1970) In the heart adrenaline increased the cyclic AMP content in the Ca^{++} poor muscle but failed to activate phosphorylase *a* (Drummond Duncan and Friesen 1965)

In the Ca^{++} poor intestinal smooth muscle both the cyclic AMP content and the phosphorylase *a* activity were reduced in comparison to normal muscle It might be considered therefore that both the action involving cyclic AMP and a direct effect of Ca were of importance in our studies In the Ca poor muscle the phosphorylase *a* activating effect of isoprenaline and cyclic AMP was however not significantly changed (Fig 5) which indicates that the changes of Ca were probably of less importance for the phosphorylase *b* kinase activity in intestinal smooth muscle than changes of cyclic AMP

ATP reduction and Ca One of the most prominent effects of Ca^{++} deprivation on the metabolic actions of isoprenaline and cyclic AMP in intestinal muscle was that the reductions of ATP and CrP were blocked It has been demonstrated that contraction of smooth muscle was dependent on utilization and reduction of its ATP content (Beviz *et al* 1965) It was therefore rather surprising that the relaxing action resulting from stimulation of the adrenergic β receptors also was associated with a reduced ATP content and ATP utilization The reduction of the ATP content was however time limited and after 10 min it had ended despite the fact that relaxation was still present and that the cyclic AMP was still increased (Fig 1) The results thus indicate that isoprenaline and cyclic AMP induced a relaxation that was associated with a Ca^{++} dependent ATP utilizing process of limited duration The most probable process known to fulfil these conditions is the accumulation of Ca by the sarcoplasmic reticulum or microsomal fraction which is an initially rapid but time limited process (Ebashi and Endo 1968) It has been reported that in the sarcoplasmic reticulum from the heart muscle the accumulation of Ca in the presence of ATP is stimulated by catecholamines or cyclic AMP (Entman *et al* 1969)

The observations reported in this paper initiated a study if there was a correspond ing process in smooth muscle In a microsomal fraction from rabbit colon muscle which accumulated Ca in the pre ence of ATP this effect was stimulated by isoprenaline and cyclic AMP (Anders on and Nilsson 1972) There is thus reason to believe that the blockade of the ATP reducing effects of isoprenaline and cyclic AMP in the Ca^{++} poor muscle was due to reduced activity of an ATP utilizing Ca^{++} accumulating mechanism in colon muscle

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ture to possess By allowing the model to be controlled by the systemic blood pressure and to electrically stimulate the sinus nerves alternate comparisons could be made between the regulatory properties of the model and those of the intact biological feed back In this study it was shown qualitatively that the proportional derivating properties of the model can influence the stability of the regulating system The model can be given such properties that the systemic response to a certain forcing function will be practically the same whether the systemic pressure/neural activity feed back is being controlled by the model or by the biological reflex arc

The third study in the series comprised a quantitative analysis of the properties of the carotid sinus reflex expressed in the form of a so called Bode diagram The amplification and phase shift of the open loop were presented An interesting observation was the reduction of the bandwidth that occurred on modulation of the input signal In addition a study was made of the properties of the system in the time domain in the form of impulse function response The non linear properties were discussed and the properties of the stimulation electrodes described

Since the time distribution of the stimulation signals applied to the sinus nerves has been found to be of great importance for the effect of the stimulation a detailed analysis has been made of three different types of stimulation and their influence on the systemic blood pressure and heart rate Further information would be obtained in this way on the relationship between sinus nerve activity and systemic blood pressure and heart rate which is of interest from the aspect of circulation physiology Further these studies are relevant to CSNS (Carotid Sinus Nerve Stimulation) therapy in hypertension (see Schwartz 1969) and angina pectoris (see Braunwald *et al* 1970)

Methods

The experiments were performed on healthy mongrel dogs of both sexes weighing 10—25 kg and of ages not exceeding year Anesthesia (chloralose by continuous i infusion) was achieved and blood pressure recordings made as described by Öberg and Sjostrand (1971) Positive pressure ventilation (HFPPV) was given at a frequency of 80/min (Jonzon *et al* 1971 a) The heart rate was recorded by an instantaneous rate meter (type 2/51 Devices Sales Ltd England)

Electrodes

In order to obtain effective stimulation with impulses of low amplitude platinized platinum electrodes (platinum black) were used throughout The low amplitudes minimized undesired spread of the stimulation current into the laryngeal muscles The magnitude of the electrode impedance of platinized platinum is relatively independent of the frequency With the geometrical form used in these studies the electrode impedance was 500—800 ohms

Types of stimulation

Fig 1 shows the precordially recorded ECG the pressure in the upper part of the descending aorta (ASP) and the simultaneously recorded pressure in the carotid sinus (ISP) The figure also illustrates the 3 different types of stimulation studied constant frequency stimulation (A) and 2 types of ECG triggered impulse bursts (B and C) where the impulses in the type B burst had a constant frequency while those in the type C burst had a linearly increasing frequency The duration between the impulses at the end of the burst in type C was longer

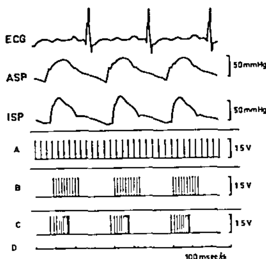


Fig 1 The time relationships between ECG systemic blood pressure (ASP) and intrasinusal pressure (ISP) in the experimental animal and the three forms of stimulation—constant frequency stimulation (A) impulse bursts with a constant frequency within the burst (B) and impulse bursts with a modulated frequency within the burst (C)

than the values given in the literature for the refractory period of the fibres of the sinus nerve The impulse burst had a duration of 150 ms The number of stimulation impulses per cardiac cycle was the same in cases A B and C The effect of stimulation by types A B and C on the arterial systemic blood pressure and on the heart rate was studied

Analysis of data

The data obtained for blood pressure and heart rate were analysed as illustrated in Fig 2 The figure shows schematically the blood pressure response to electrical stimulation of the sinus nerves as has been described previously (Öberg and Sjostrand 1969 b 1971) The analogous course of the heart rate is given in the upper part of the figure and in the lower part the systolic and diastolic components of the systemic blood pressure are given as per cent of the respective initial values before electrical stimulation of the sinus nerves The designations given in the figure represent the following

P represents the initial maximal systolic blood pressure reduction in relation to the initial systolic blood pressure before stimulation P_d is the corresponding relationship for the diastolic blood pressure

P_m is a type of mean value of the obtained systolic blood pressure reduction when the initial oscillatory course has ended P_{md} represents the corresponding reduction for the diastolic pressure The percentage blood pressure reduction is given in relation to the respective levels before stimulation

ΔP represents the interval (in per cent of the systolic pressure before stimulation) within which the systolic blood pressure variations take place during the period when P_m is calculated ΔP_d is the corresponding interval concerning variations in the diastolic blood pressure

HR is the maximal reduction in heart rate taking place during the initial oscillatory course

HR_m is a type of measure of the mean reduction in heart rate during the time for which P_m and P_{md} are calculated

ΔHR represents the variation in heart rate during the time for which HR_m is calculated and is given in per cent of the heart rate before stimulation

Experimental procedures and results

During the experiments in which clamping of the common carotid artery was performed simultaneous with sino venous shunting (Öberg and Sjostrand 1969 a) the intrasinusal pressure was of an order of magnitude of 30—40 mm Hg and only slightly pulsating When the common carotid artery was not clamped the intra-

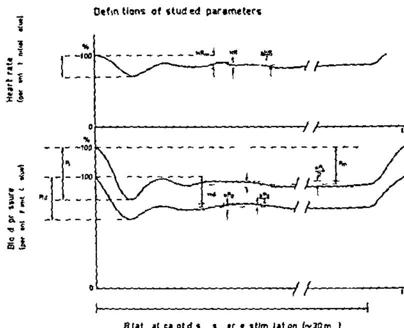


Fig 2 Definitions of the studied parameters

sinusal pressure had a course analogous to that in Fig 1 this experimental condition is referred to hereinafter as unclamped

Electrical stimulation of the sinus nerves was accomplished via two isolated stimulators The duration of the stimulation impulses was 0 5 ms The stimulators were controlled by a system with the following construction The dog s ECG was amplified and the QRS complex in the ECG was applied after pulse shaping to a delay circuit The delay of the impulse burst was varied in relation to the ECG This arrangement was used in order to place the impulse burst such that it coincided in time with systole The output signal of the delay circuit controlled a function generator which during a time period of 150 ms generated impulses of the desired frequency and controlled the 2 stimulators The number of impulses for each burst was counted by an electronic counter (Racal 835 Racal Ltd England) The impulse burst (stimulation types B or C) was applied to the sinus nerves analogously with Fig 1

Procedure and results I Dependence of heart rate and blood pressure on amplitude of stimulation impulses with stimulation type B

The effect of the amplitude of the stimulation impulses on the parameters described in Fig 2 was studied These experiments with stimulation type B (25 pulses/cardiac cycle) were performed on 16 dogs with normal sinus nerve activity (unclamped) Stimulation impulses with amplitudes of 0—3 5 V were fed to the electrodes applied

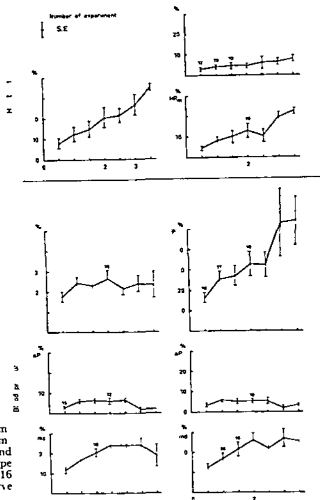
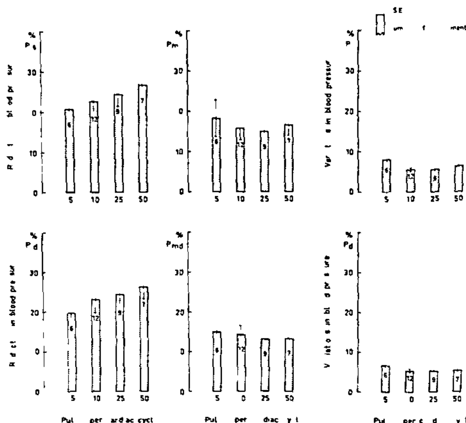


Fig 3 The influence of the amplitude of the stimulation impulses on the heart rate and blood pressure in stimulation type B (25 imp/cardiac cycle) in 16 dogs with normal sinus nerve activity (unclamped)

to the sinus nerves The effect of stimulation type B was studied during 5 minute periods The results are presented in Fig 3

The initial reduction in heart rate HR increased with increasing stimulation amplitude and this was also true for the mean heart rate reduction HR_n The variations in heart rate (ΔHR) during the period following the initial oscillatory course showed a tendency to an increase with increasing amplitude

The initial reduction in systolic blood pressure (P) did not appear to be influenced by *the amplitude of the stimulation impulses while the initial reduction in* diastolic blood pressure (P_d) on the other hand increased with increasing amplitude The mean systolic (P_m) and diastolic (P_{m1}) blood pressure reductions increased linearly within the amplitude range 0 5—2 V and the variation in systolic (ΔP) and diastolic (ΔP_1) blood pressure was approximately the same within the range 1—2 5 V



Fi 4 C mpari on bet een the blood pressure reducing and blood pres ure stabilizing effects respectivel of 5 10 5 and 50 stimulation imp /c rdiac cycle in stimulation type B in 5 d gs whose endogenous sinus nerve activity had been eliminated (clamped)

Procedure and results II Dep nd nc of blood pr sure on numb r of stimulation impulse per ardiac cycle with stimulation typ B

In Fig 4 the reduction in blood pressure is related to the number of impulses per burst The studies compri ed stimulation with 5 10 25 and 50 imp per burst and were carried out on 5 dogs in which the endogenous sinus nerve activity had been eliminated (clamped) In each individual ca e measurements were made during 300 cardiac cycles The amplitude of the stimulation impul es was 1 5 or 2 V

The initial systolic (P) and diastolic (P) pressure reduction both increased *with increasing numbers of impul es in the burst The reduction of the systolic* (P_m) and diastolic P_{md}) blood pressure was independent of the number of stimu lation impul es applied to the sinus nerves per cardiac cycle

Procedure and result III The blood pressure with stimulation types A and B

Essentially the same experimental arrangement as described above was used A compari on between the effect on the blood pressure of stimulation with impul es of constant frequency Fig 1 A) and of stimulation with impul e bursts (Fig

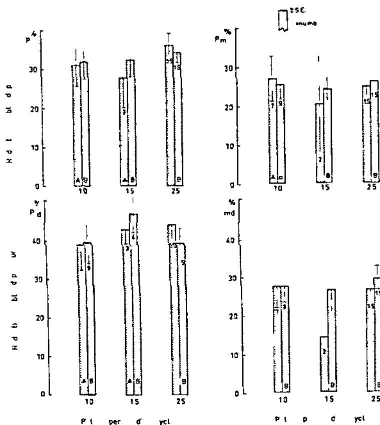


Fig 5 Comparative studies of the effects of stimulation types A and B at 10 15 and 25 imp/cardiac cycle in 12 dogs with eliminated endogenous sinus nerve activity ("clamped") The effects on the heart rate can be seen to the right in Fig 8

1 B) was made in 12 dogs whose endogenous sinus nerve activity had been eliminated ("clamped" Fig 5) and in 2 dogs with normal sinus nerve activity ("unclamped" Fig 6) The studies comprised 10 15 and 25 imp per cardiac cycle The amplitude of the impulses was 1 5 or 2 V

P_{is} and P_{id} and also P_m and P_{md} were greater under "clamped" (Fig 5) than "unclamped" conditions (Fig 6) which is in agreement with findings in previous studies in this series and with the results of other authors

In Fig 5 the initial blood pressure reductions (P_s and P_d) remained about the same or increased slightly with increasing number of impulses per cardiac cycle P_m and P_{md} showed no appreciable differences with the two different types of stimulation (in 8 of the cases presented in the figure stimulation type B gave a slightly greater blood pressure reduction than type A in 1 case the reduction was the same and in the remaining 3 cases type B gave a slightly smaller reduction)

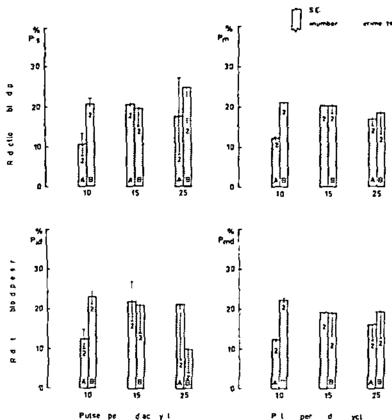


Fig 6 Comparative studies of the effects of stimulation types A and B at 10 15 and 25 imp/cardiac cycle in 7 dogs with normal sinus nerve activity (unclamped) The effects on the heart rate can be seen to the left in Fig 8

In dogs with normal sinus nerve activity (unclamped) the induced activity of stimulation type A or B (Fig 6) is superimposed upon the normal activity in the sinus nerves At 10 stimulation impulses per cardiac cycle the blood pressure reductions P_{s} and P_{d} and also P_{m} and P_{md} were greater with stimulation type B At 15—25 imp per cardiac cycle the differences between the two types of stimulation were negligible (*cf* also before bleeding in Fig 9)

In the series of experiments presented in Fig 4 5 6 7 and 9 the greatest reduction and the smallest initial decrease in blood pressure on stimulation were found as a rule within the range of 5—10 stimulation impulses per cardiac cycle This means that the reduction of the arterial blood pressure will not be greater for a larger number of stimulation impulses per cardiac cycle

Procedure and results II The blood pressure with stimulation types B and C

A comparison between the effect of stimulation of the sinus nerves with 2 different types of ECG triggered impulse bursts B and C (as in Fig 1) on the blood pressure was made

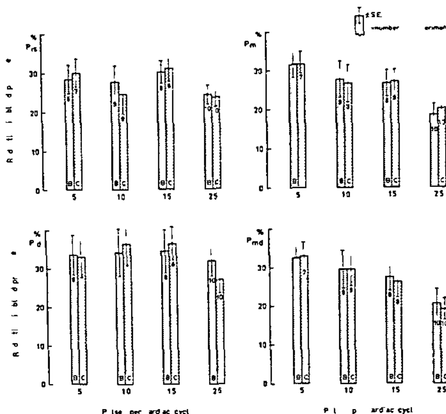


Fig 7 Comparative studies of the effects of stimulation types B and C at 5 10 15 and 25 imp/cardiac cycle in 10 dogs with normal sinus nerve activity (unclamped) The effects on the heart rate can be seen to the left in Fig 8

In those studies in which modulation took place within the impulse burst (cf Fig 1 C) the system was complemented in accordance with procedure I above such that the signal evoked by the delay circuit controlled a function generator which produced a ramp with a variable slope which was fed to a VCG generator The signal thus obtained controlled the two stimulators The impulses were counted by an electronic counter The experiments were performed on 10 dogs with normal sinus nerve activity (unclamped) The amplitude of the stimulation impulses was 1 5 or 2 V

No definite differences between stimulation types B and C were noted at the studied number of impulses per burst (Fig 7) P and P_1 were slightly smaller than in Fig 5 (clamped) A tendency to a reduced effect with increasing number of impulses was observed for P and P_{m1} Taking into account the size of the material a statistically significant difference was obtained for P_m between 5 and 10 stimulation imp ($p < 0.1$) and between 15 and 25 imp ($p < 0.02$) in paired observations both for stimulation types B and C

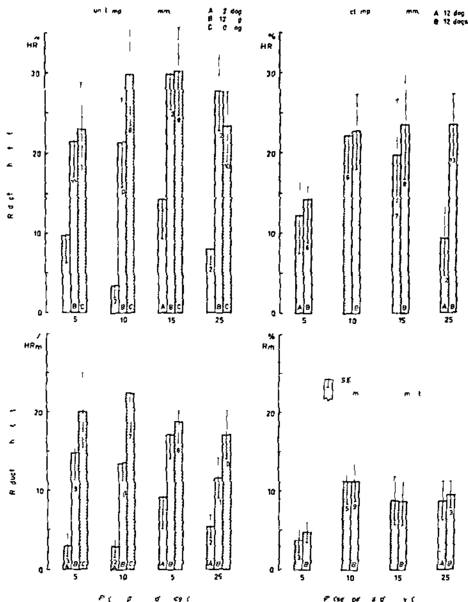


Fig 8 Comparative studies of the effects of stimulation types A B and C on the heart rate at 5 10 15 and 25 imp/cardiac cycle The left side of the figure gives the results from dogs with normal sinus nerve activity (unclamped) and the right side of the figure gives the results from dogs with eliminated endogenous sinus nerve activity (clamped) Blood pressure data for the unclamped dogs are given in Fig 6 and 7 and for the clamped dogs in Fig 5

Results III and IV The heart rate with stimulation types A B and C

Fig 8 shows the effect of stimulation types A B and C on the heart rate All three types were studied in dogs with normal endogenous sinus nerve activity (un

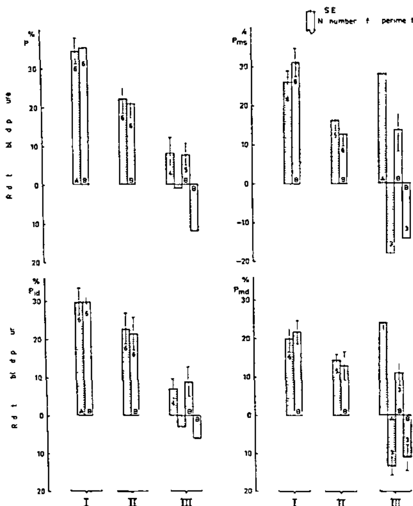


Fig 9 Comparative studies of the effects of stimulation types A and B at 15 imp/cardiac cycle on the systemic blood pressure in dogs with normal sinus nerve activity (unclamped) The studies were performed on 6 dogs under chloralose anesthesia both with a normal blood volume (I) and after exsanguination to two different systolic blood pressure levels—1?5 (II) and 75 (III) mm Hg

clamped left side of figure) and type A and B in dogs in which the endogenous sinus nerve activity had been eliminated (clamped right side of figure)

It is seen that the effect on the heart rate is dependent upon the type of stimulation used In general stimulation type A had a smaller effect on the heart rate than B and C and was independent of the number of impulses per cardiac cycle Type C however showed a tendency to induce a greater effect on the heart rate than type B Further type B gave a greater HR_m in unclamped than in clamped ditions

TABLE I The occurrence of occasional spontaneous breaths during high frequency positive pressure ventilation in CSNS

Stimulation type	Common carotid arteries unclamped			Common carotid arteries clamped		
	Number of dogs	Number of expts	Spontaneous breaths (% of number of expts)	Number of dogs	Number of expts	Spontaneous breaths (% of number of expts)
A	6	16	31	15	33	18
B	14	49	10	15	43	2
C	8	33	12	—	—	—

Procedure and results V The blood pressure on massive hemorrhage (stimulation types A and B)

Six dogs were studied with respect to the effect of stimulation types A and B (15 stimulation impulses per cardiac cycle) on the systemic blood pressure before and after exsanguination to two different systolic blood pressure levels ie about 125 and 75 mm Hg The animals had normal sinus nerve activity (unclamped) The results are presented in Fig 9 where in all cases the blood pressure reduction is calculated in per cent of the initial pressure before electrical stimulation of the sinus nerves

Before the hemorrhage a large decrease in blood pressure both systolic and diastolic was noted on commencement of the sinus nerve stimulation (P_{si} and P_{di}) As in other experimental serie the decrease was somewhat smaller after the initial phase After exsanguination to a systolic blood pressure of 125 mm Hg the blood pressure reduction resulting from the sinus nerve stimulation was smaller At a systolic blood pressure of 75 mm Hg a reduction of both the systolic (P_{si}) and diastolic (P_{di}) pressure was noted initially in most cases after the initial phase under these conditions (see P_{sm} and P_{md}) there was a rise in blood pressure in most dogs despite continued sinus nerve stimulation

Procedure and results VI Spontaneous respiration during high frequency positive pressure ventilation and sinus nerve stimulation stimulation types A B and C

In the chloralose anesthetized animals (clamped or unclamped) subjected to the above studies occasional (1–5) spontaneous breaths were sometimes noted during the course of the sinus nerve stimulation (30 min) in spite of the presence of normal blood gas conditions Usually no spontaneous respiration occurs with this type of positive pressure ventilation (Jonzon *et al* 1971 a) and the rhythmic variations in the central sensitivity to afferent sinus nerve activity (Koepchen *et al* 1961) is probably diminished The occurrence of occasional spontaneous breaths on sinus nerve stimulation is shown in Table I

Discussion

Constant frequency stimulation

In a preliminary series of experiments on clamped dogs a study was made of the blood pressure reducing effect of stimulation of the sinus nerves with impulses of constant frequency during a 30 min period Frequencies corresponding to 5—100 imp/cardiac cycle were studied here and it was found that no further blood pressure reduction could be produced by a greater number of impulses than 50 imp/cardiac cycle The studies reported in this paper were therefore limited to impulse numbers of 50 per cardiac cycle or less Amplitudes of 1 5 or 2 V were used throughout

Other authors have studied the blood pressure reduction at different frequencies of stimulation Myers *et al* (1968) studied the interval between the stimulation impulses in relation to the decrease in blood pressure and found an increasing blood pressure reduction with decreasing intervals between the impulses (*i e* increasing frequency) The maximal reduction was obtained at the highest frequency studied *i e* 200 Hz Over a large range the blood pressure reduction was relatively independent of the frequency The reason that the maximal reduction in the study of Myers *et al* was reached at a rather higher frequency than in the present study may be due to differences in the electrode impedances (platinum iridium and platinized platinum respectively) and electrode geometry (hook electrodes and circumferent electrodes respectively) in the two methods

The blood pressure reduction at a constant impulse voltage time area was also studied by Myers *et al* (1968) The blood pressure response was given in a first approximation as a function of the product of voltage and time

Bilgutay and Lillehei (1965) also investigated the effect of the stimulation frequency at a constant pulse amplitude up to a frequency of 65 Hz a continuous reduction of the mean blood pressure was obtained between 65 and 100 Hz there was no appreciable change in the stimulation effect and over 100 Hz a reverse effect was noted

Schwartz *et al* (1967) have claimed that frequencies between 50 and 100 Hz (4 V 0 3 ms) give a maximal reduction of the mean blood pressure In a later study by Testerman and Schwartz (1971) the reductions in blood pressure and heart rate increased with increasing stimulation frequencies at least up to a frequency of 100 Hz An increase of the stimulation current at a constant frequency markedly potentiated the effect of the stimulation both as regards blood pressure drop and heart rate reduction The experiments in the present study were performed with low impedance electrodes and the impulse amplitudes used therefore gave a relatively high stimulation current compared with the same nominal values reported by other authors with consequent effective stimulation (a large blood pressure reduction at low frequencies) This result stresses the important of effective electrodes in

therapy which has been pointed out previously by Myers *et al* (1968) and Testerman *et al* (1971)

Stimulation pulse amplitude

The results in Fig 3 offer direct guidance in the choice of stimulation amplitude with respect to change in blood pressure and heart rate A low stimulation amplitude gives small reductions in both blood pressure and heart rate while high amplitudes greater than 3 V with the electrodes used give a pronounced initial bradycardia and side reactions in the form of local stimulation effects Stimulation with high amplitudes did not result in spontaneous hyperventilation as was described by Drews *et al* (1968) As is evident from Fig 3 no further blood pressure reduction (P_{ms} P_{mi}) was obtained on stimulation with impulse amplitudes greater than 2 V while the heart rate could be reduced further within the range 2—3 5 V (but with initial bradycardia and a marked increase of the pulse pressure)

Analogous results indicating dependence of the systolic and diastolic pressures on the stimulation amplitude (stimulation impulses 80 Hz duration 0 5 ms) were obtained in normotensive (and acutely hypertensive) dogs by Drews *et al* (1968) The results in Fig 3 motivated a stimulation amplitude of 1 5 or 2 V which was tested initially in each individual experiment Differences in the electrode material between the present studies (platinized platinum) and other investigations (*e g* Drews *et al* 1968 and Schwartz 1969) make it difficult to compare our results with those of other authors with respect to either the amplitude of the stimulation pulses or the number of stimulation impulses per cardiac cycle

The results are of interest with respect to therapeutic CSNS They indicate the possibility of diminishing risks and side effects in this form of therapy by limiting the amplitude of the stimulation signals

The greater reduction of the heart rate with increasing stimulation amplitude both initially during sinus nerve stimulation (HR_i) and after the initial oscillatory course (HR_m) is an expression of an alteration of the autonomic influence on the heart (heart rate stroke volume/contractility) The effect on the blood pressure and heart rate induced by stimulation type A (see below) is in accordance with the results of Griffith and Schwartz (1964) Drews *et al* (1968) and Vatner *et al* (1970) Cardiac output determinations in anesthetized dogs on CSNS with constant frequency (Drews *et al* 1968 Resnikoff *et al* 1969) have shown that the cardiac output remains practically unchanged and that the total peripheral flow resistance decreases during CSNS in normotensive acutely hypertensive and renal hypertensive dogs Vatner *et al* (1970 b) in studies on conscious dogs determined the cardiac output and the peripheral flow resistance at rest and during exercise and found that the cardiac output at rest was not affected by 30 s of CSNS

The longer filling time during diastole in the present study probably explains the fact that both P_{si} and P_{mi} increase with increasing stimulation amplitude (due to greater reduction of the heart rate) while the increase in the stroke volume is responsible for the greater pulse pressure during sinus nerve stimulation

Impulse train stimulation

On stimulation with a constant frequency (stimulation type A) a maximal reduction in blood pressure was obtained in this study at 5—10 imp/cardiac cycle and therefore frequencies of 5 10 15 and 25 imp per burst (and cardiac cycle) were studied in ECG synchronized intermittent stimulation As is evident from Fig 4 for stimulation type B no further blood pressure reduction (P_m and P_{m1}) was obtained with a greater number of impulses than 5 per cardiac cycle while the initial blood pressure reduction on the other hand increased with an increasing number of impulses Similar results were obtained in the series of experiments presented in Fig 5 where no further blood pressure reduction (P_m and P_{m1}) with a greater number of impulses than 10 per cardiac cycle with otherwise similar experimental conditions (clamped), was obtained

Central evaluation of impulse pattern

Testerman *et al* (1971) in agreement with other authors have found that it is impossible to isolate the effect of sinus nerve stimulation on the blood pressure and on the heart rate by varying the stimulation parameters—amplitude duration current density and frequency In previous studies in this series we have found that the time distribution of the stimulation signal influences the properties of the blood pressure regulating system The degree of influence in the frequency domain has been expressed (Öberg and Sjostrand 1971) as changes in the amplification of the blood pressure regulating system If different components of the system (systemic blood pressure heart rate cardiac output etc) are studied changes related to the time distribution of the forcing function must also be found in these — In the comparisons between stimulation types A and B it can be seen in Fig 5 6 and 8 that the effect on the systemic blood pressure is approximately the same with the two types while the change in heart rate differs greatly between them Similarly minor differences can be distinguished between types B and C with regard to the effect on the heart rate but not for blood pressure reduction

No appreciable differences were observed in the blood pressure reduction with stimulation types B and C (Fig 7) As has been pointed out previously by Öberg and Sjostrand (1971 p 107) this finding indicates that the central discrimination of afferent information is limited

The effect of CSNS in massive hemorrhage exemplifies the multi input character of the blood pressure regulating system *i e* that the control is dependent upon information from many different sensory systems The results obtained in the experiments with CSNS in hemorrhagic conditions (Fig 9) can probably therefore be explained by a central mechanism Through the multi input nature of the system the function of the carotid sinus reflex will be eliminated by degrees in a condition of hemorrhage due to the dominance of other vasoregulatory mechanisms that enter into function during the hemorrhage This holds to an equally high degree for stimulation type A as for type B

The heart rate on CSNS

Rhythmic changes of the central sensitivity to electrical stimulation of the sinus nerves in relation to respiration in anesthetized dogs especially as regards the effects on heart rate has been described by Koepchen *et al* (1961)

The changes in blood pressure and heart rate described by Griffith and Schwartz (1964) and Drews *et al* (1968) in anesthetized dogs and by Vatner *et al* (1970 a b) in conscious dogs with CSNS at constant frequency (usually about 50 Hz) show good agreement The effects on the blood pressure and heart rate observed in the present study with artificial respiration (HFPPV) and with CSNS of type A (Fig 6 and 8) do not deviate from the findings of other authors

The heart rate is reduced to a greater extent with the ECG synchronized stimulation types B and C than with type A (constant frequency stimulation) see Fig 8 The ECG synchronized stimulation types (B C) probably therefore affect the balance between the sympathetic and parasympathetic systems to a higher degree than type A Further Richter *et al* (1970) have shown that the inhibition of the efferent sympathetic activity is much more pronounced on CSNS with impulse bursts than on CSNS with a constant frequency The results of this investigation therefore suggest that it may be possible within certain limits to induce a selective and graded effect on the heart rate by a suitable choice of stimulation signal This is of interest both from a circulation physiological aspect and from the aspect of CSNS itself In the treatment of angina pectoris with CSNS a graded effect on the heart rate would in some cases be an advantage

Ventilation on CSNS

Seller *et al* (1968) have demonstrated that continuous frequency stimulation of the sinus nerves in chloralose anesthetized dogs intensifies the respiratory grouping of sympathetic activity (spontaneous respiration)

Drews *et al* (1968) found that on experimental stimulation with a constant frequency (tubular electrodes) with impulse amplitudes greater than 4 V and *durations longer than 0 5 ms the animal hyperventilated*

Epstein *et al* (1969) found no change of the respiration in investigations of the circulatory and ventilatory effects of CSNS in man No effects on the respiration were observed in the animal studies and clinical applications of CSNS reported by Schwartz (1969) and Braunwald *et al* (1970)

In the present study occasional spontaneous breaths were noted during stimulation (30 min) of the carotid sinus nerves A difference between stimulation type A and types B and C was found in this respect spontaneous breaths during positive pressure ventilation being observed more often when stimulation of type A was used Changes of the afferent baroreceptor information may therefore alter the interrelated functions of the cardiovascular and respiratory centers

Whether the differences between stimulation types A B and C with respect to the occurrence of spontaneous breaths are due to central regulatory mechanisms

CSNS of types A, B and C (the maximal effect of electrical sinus nerve stimulation was obtained in these studies at only 5—15 stimulation imp/cardiac cycle) The figure shows the mean values for the systemic blood pressure (systolic and diastolic) and the heart rate of steady state both without sinus nerve stimulation (ASP and HR) and with CSNS of types A B and C at 15 stimulation imp/cardiac cycle, in dogs with normal sinus nerve activity With electrical sinus nerve stimulation the systolic and diastolic mean levels (P_m and P_{mf}) and the mean heart rate (HR_m) are given

As is clear from Fig 10 both blood pressure and heart rate are in the unstimulatd case well above what is normally found in resting healthy dogs The dogs in this study were normotensive and healthy before general chloralose anesthesia but during anesthesia the general *reflex* excitatory effects of *chloralose normally made the* experimental animals hypertensive due to an increased sympathetic dominance (for review see Balis and Monroe 1964)

As mentioned previously the order of size of the mean systolic and diastolic blood pressure reductions is the same in all three types of CSNS studied The effect on the heart rate is however more marked with types B and C This suggests that types B and C affect the central circulatory regulation differently from type A (*cf* also studies by Richter *et al* 1970 on the sympathetic outflow on electrical stimulation of the sinus nerves)

The greater heart rate reduction induced by stimulation types B and C exemplified in Fig 10 is of interest in the question of therapeutic CSNS both in hypertension angina pectoris and supraventricular tachycardia (Sjostrand 1971) In all cases all 3 stimulation types give a reduction of the systolic and diastolic arterial blood pressure This means a decrease in the pressure work of the heart and consequently *a reduction of the oxygen requirement of the cardiac muscle The reduced* heart rate resulting from CSNS also decreases the oxygen requirement of the cardiac muscle According to Drews *et al* (1968) and Resnicoff *et al* (1969) the reduced heart rate in anesthetized dogs only leads however to a negligible reduction of the cardiac output and in conscious dogs CSNS at a constant frequency gave no decrease of the cardiac output (Vatner *et al* 1970 b) If the results of the above authors can be applied to the present studies the cardiac output will remain unchanged on CSNS of type A (constant frequency) Whether stimulation types B and C with their equal effects to type A on the blood pressure but greater effect on the heart rate lead to decrease of the cardiac output remains to be determined in further experimental studies

Conclusions

The results of this study show that the types of stimulation used have approximately equal effects with respect to blood pressure reduction but can differ in their effects on various parameters in the circulatory system *e g* the heart rate This is of interest in view of the changes in the sinus nerve activity in hypertension reported by

Kubicek (1953) McCubbin *et al* (1956) and Kezdi (1967) and others and the vessel wall and receptor changes found by Abraham (1967) and Jones *et al* (1967) which in turn affect the impulse pattern Further studies with quantitated sinus nerve stimulation can therefore be expected to give information concerning central interpretation of the afferent information and thereby increase our knowledge of both the normal and pathological blood pressure regulation.

The present results also illustrate the great importance of effective electrodes for therapeutic CSNS

A possible isolation of the stimulation effect to certain circulatory parameters is also of interest for the development of CSNS as a therapeutic method

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Content of Blood and of Extravascular Water in Cat Lungs during Changes in Total Blood Volume

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Abstract

Aarseth P and G Bo *Content of blood and of extravascular water in cat lungs during changes in total blood volume* Acta physiol scand 1972 *85* 343—352

A method has been developed which allows alterations in lung blood content and in extra vascular lung water to be studied The 2 upper lung lobes were removed separately from open chested cats The first lobe was taken for control evaluations The second one was removed when the circulatory situation had been changed Labelled erythrocytes and plasma albumin had been injected beforehand From the content of isotopes in the lung lobes their blood volume could be calculated Extravascular water was found by taking the difference between wet and dry weight of lung tissue and correcting this difference for the blood content in that lobe. When total blood volume was reduced by a small bleeding lung extravascular water content was reduced and there was also a small reduction in lung blood volume After a larger bleeding lung blood content was markedly reduced while the extravascular lung water content was then somewhat increased Augmentation of total blood volume by a blood transfusion led to an increase in lung blood volume but to a decrease in extravascular lung water content

The pulmonary blood volume in the rat can change markedly upon acute moderate changes in total blood volume (Aarseth 1970 Aarseth 1971) The extravascular water content of the rat lungs decreased during acute hypovolemia as well as during acute hypervolemia. The method used in those experiments was injection of isotope labelled erythrocytes and plasma albumin followed by subsequent rapid freezing of the rats in liquid nitrogen Lung blood volume could then be estimated from the isotope content in the excised frozen lungs From the blood volume and the total weight of the lungs also changes in extravascular water content could be arrived at

The present experiments have been undertaken in order to see how the pulmonary vascular bed in another animal than the rat reacts to acute hypo and hypervolemia With cats as experimental animals the method of freezing the whole animal could not be used Instead the 2 upper lung lobes were removed separately from open chested animals where isotope labelled erythrocytes and plasma albumin had been injected beforehand The blood content and the water content of lobes removed in different experimental situations could then be compared Some remarks on the usefulness of this new method will also be included in the following

Two sets of experiments will be presented In the first set was tested the reaction of the pulmonary vascular bed in the cat to a moderate acute blood loss and also to a blood transfusion Here care was taken to induce changes in total blood volume equal to those used in the previous experiments in the rat (Aarseth 1970 1971)

Secondly the acute changes in the fluid compartments in the cat lung during a more marked bleeding was studied For this purpose one used the technique and the bleeding arrangement which has been developed and thoroughly studied by Bo and Hognestad (1971) Some present evaluations of the more longterm changes in pulmonary fluid compartments during such a hypotensive period are presented elsewhere (Aarseth and Bo 1972)

Methods

Cats weighing 2 0—4 2 kg were used They had free access to food and water until the experiments started 30—45 mg/kg Nembutal (Abbott®) was given i p

The main experimental procedure was as follows In open-chested animals on positive pressure ventilation snares were placed around the hilus of the 2 upper lobes Using a special instrument these snares could be rapidly tightened and the lobe circulation thereby suddenly stopped A portion of labelled erythrocytes and plasma albumin was injected in the femoral vein One of the upper lobes was removed and simultaneously a blood sample was taken from the femoral artery The blood content and extravascular water content in the lobe was calculated and used as control values for this particular animal Then total blood volume was anged by bleeding or transfusion and the second lobe was removed Again a blood sample taken from the femoral artery Care was always taken to stop the lobe circulation at the of an expiration

Ventilation was performed with a positive pressure pump and the volume was regulated so s to keep the pH of arterial blood within narrow limits (7 35—7 45) End expiratory pressure was kept at 1 cm H_2O The lungs were given a moderate hyperinflation at intervals of 30 min

Labelling of blood by radioisotopes Erythrocytes were labelled with ^{1}Cr Three ml of whole blood was added to 1 ml of isotonic sodium citrate and centrifugated at 800 × g for 10 min The supernatant was discarded and 0 1 mCi ^{51}Cr as Na_2CrO_4 was added Incubation was performed at room temperature for 30 min under constant shaking The erythrocytes were then washed 4 times in heparinized saline and each time centrifugated for 10 min at 800 × g To the erythrocytes was added an equal volume of isotonic saline containing 0 01 mCi ^{12} I human serum albumin/ml A weighed amount of this mixture was injected through the catheter in the femoral vein 10 min before the first lobectomy The radioactivity in 2 weighed samples of the same mixture was evaluated

Calculations The lung lobes were frozen in liquid nitrogen and divided between several test tubes in such a way that there was not more than 2 g tissue in any tube Two blood samples 2 samples of the injected isotope mixture and the tissue samples were counted in a 3 Channel Auto Gamma Spectrometer (Model 50?? Packard Instrument Co) At the gain and discriminator settings used no ^{12} I activity was measured in the channel set for ^{51}Cr counting The ^{1}Cr activity which was counted in the ^{1} I channel was corrected for Knowing the amount and the radioactivity of the injected isotope mixture the weights the hematocrit the ^{12} I and ^{51}Cr activity of each blood sampl total erythrocyte and plasma volumes could be calculated

All the tissue samples the blood samples and 2 plasma samples were weighed and then dried for several days at 75 C until the weight became stable From the obtained data the lobe erythrocyte and plasma volumes as well as the extravascular lung water content were calculated from the following equations

Erythrocyte volume of the lobe (E 1) =

$$\frac{(\text{cpm of } ^{1}\text{Cr in lobe}) \times (\text{blood sample weight}) \times (\text{Hct})}{(\text{cpm of } ^{1}\text{Cr in blood sample}) \times 1\ 05}$$

Plasma volume of the lobe (Pl 1) =

$$\frac{(\text{cpm of } ^{12}\text{ I in lobe}) \times (\text{Blood sample weight}) \times (1 - \text{Hct})}{(\text{cpm of } ^{12}\text{ I in blood sample}) \times 1\ 05}$$

Specific plasma dry weight (Pl_{DW}) in g/ml =

$$\frac{(\text{Dry weight of plasma sample}) \times 1.03}{\text{Wet weight of plasma sample}}$$

Specific erythrocyte dry weight (E_{DW}) in g/ml =

$$\frac{[1.05 \times (\text{Dry weight of blood sample})] - [Pl_{DW} \times (\text{Blood sample weight}) \times (1 - \text{Hct})]}{(\text{Blood sample weight}) \times \text{Hct}}$$

Extravascular wet weight of lung tissue (EV_{WW}) = Wet weight of lung lobe $-$ ($E_{vol} \times 1.07 + Pl_{vol} \times 1.03$)

Extravascular dry weight of lung tissue (EV_{DW}) = Dry weight of lung lobe $-$ ($E_{vol} \times E_{DW} + Pl_{vol} \times Pl_{DW}$)

Extravascular water volume = $EV_{WW} - EV_{DW}$

1.03, 1.05 and 1.07 are here the specific weights of plasma, blood and erythrocytes respectively. Hct is the measured hematocrit in the blood sample from the large peripheral vessel. All fluid volumes in the lobe were expressed as ml/g extravascular dry weight.

Pulmonary hematocrit was calculated as pulmonary erythrocyte volume/(pulmonary erythrocyte volume + pulmonary plasma volume) and expressed as % of the measured hematocrit in blood from large vessels.

Total lung tissue dry weight. After the 2 experimental lobectomies had been performed, the rest of the lung was removed in some animals. Their contents of radioisotopes were found and then the lungs were dried. The dry weight was then corrected for the calculated dry weight of the blood within the lung so that the total net dry weight of lung tissue was found.

Wilcoxon's two sample test has been used for evaluation of significance of differences between groups. When nothing else is said a two-sided test is being used.

Results

In the first set of experiments the immediate effects on pulmonary fluid volumes of moderate changes in total blood volume were studied. Three groups of animals were used. In all groups the 2 lobectomies were performed with 10 min interval. One group served as a control group. In a second group the animals were exposed to a blood loss. After the first lobectomy bleeding was started at a rate calculated to give a blood loss of about 12 % of the total blood volume. The second lobe was then removed. The blood withdrawn in these experiments was again used for transfusions to the animal in a third group. A compatibility test was performed between the blood from the donor and the blood from the recipient before such a transfusion.

In open chested animals it is necessary to carry out moderate hyperinflations at intervals in order to prevent a gradual reduction in tidal volume. Hyperinflations do however induce some moderate pressure and flow changes in the pulmonary circulation. It then takes about 10—15 min before these parameters are stabilized at preinflation levels again. In this series of experiments the last hyperinflation was therefore carried out 30 min before the first lobectomy. Both lobes were thus removed in a period where there should be no remaining vascular effects of the hyperinflation.

In 4 control cats (mean b.w. 3.0 kg ± 0.9 S.D.) the second lobectomy was performed 10 min after the first one but no bleeding was carried out inbetween. In 3 of these animals the extravascular water content was larger in the second lobe than in the first one, while there was no change in this parameter in the fourth animal (mean increase 3 %). The blood content in the second lobe showed an increase in 2 animals, a decrease in 1, while there was no change in the fourth animal (mean

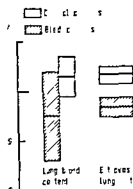


Fig 1

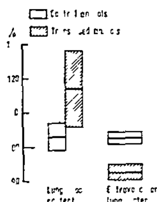


Fig 2

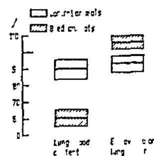


Fig 3

Fig 1 Changes in lung blood content and in extravascular lung water in cats exposed to a moderate blood loss Actual reduction achieved 4 % of total blood volume Two lung lobes were removed separately one as a control, the other 10 min later and after the blood loss The volumes found in the second lobes are expressed as % of the volumes found in the first lobes The same procedure was carried out in non bled control animals in order to find the effect of time and of the first lobectomy on the remaining lung For further details of procedure see text The mid lines and areas on both sides give mean values ± S E

2 Changes in lung blood content and in extravascular lung water in cats in which the blood volume had been increased by 17 by a transfusion of whole blood For details rocedure see Fig 1 and text Mid lines and areas on both sides give mean values ± S E

Fig 3 Changes in lung blood content and in extravascular lung water in cats exposed to an effective blood loss of 34 % of their total blood volume Two lung lobes were removed separately one as control the other 30 min later and after the blood loss The volumes found in the second lobe are expressed as % of the volumes found in the first lobe The same procedure was carried out in non bled control animals in order to find the effect of time and of the first lobectomy on the remaining lung For further detail of the procedure see text The mid lines and areas on both sides give the mean values ± S E

increase 3 %) The mean changes are shown in Fig 1 The hematocrit did not change during the experiment The distribution volume for the isotopes increased by only 2 % indicating a small leakage of ^{131}I albumin from the circulation.

Effects of a moderate blood loss In a test group of 5 cats (mean b w 2 9 kg ± 0 2 S D) the total blood volume was reduced by 12 % of its initial value When blood content per net dry tissue weight was compared in the lobe removed before and in the one removed after the blood loss a decrease was found in 4 animals and an increase in 1 (mean decrease 5 %) The extravascular water content was reduced in 4 and increased in 1 animal with a mean reduction of 3 2 % (Fig 2) The pulmonary hematocrit was found to be 69 3 % and 67 % of large vessel hematocrit in the first and second lobe respectively

The distribution volume for the isotopes increased by 10 % during the 10 min between the two lobectomies and the hematocrit of large vessel blood fell from 34 6 to 32 6

Some effects of the bleeding are revealed when the changes in the second lobe in the control group are compared with the changes in the second lobe in the bled group (Fig 1) After the bleeding there is hardly any change in lung blood content. The reduction in extravascular lung water content agrees with what has been found in lungs of bled rats (Aarseth 1970) When tested with one sided Wilcoxon test this reduction in extravascular lung water content was significant ($p = 0.033$)

Effects of an increase in total blood volume In 4 cats (mean b w 2 1 kg ± 0 2 S D) the total blood volume (mean 131 ml ± 29 S D) was increased by a transfusion of whole blood The mean increase in total blood volume amounted to 16 % In these animals the lung blood content had increased by 17 % when the second lobe was removed after 10 min The extravascular lung water content decreased in all animals (mean decrease 7 2 % (Fig 2)) Again qualitatively similar changes to those seen in transfused rats (Aarseth 1971) was found The reduction in extravascular lung water content from the first to the second lobes in the present experiments was highly significant when tested with a one sided Wilcoxon test ($p = 0.008$) The pulmonary hematocrit was reduced from 75 1 to 70 9 % of the hematocrit found in peripheral blood

Effects of a large blood loss The acute effects of a larger bleeding was studied in another series of experiments where the animals were exposed to a longlasting period of hemorrhagic hypotension The experimental procedure for these experiments is described elsewhere (Aarseth and Bø 1972) The techniques for evaluating pulmonary fluid volumes were however exactly the same as described above. The animals used in these experiments had catheters introduced into their pulmonary artery and left auricle Also an electromagnetic flow probe was wrapped around the ascending aorta.

It is known from previous studies on a preparation of this type that the pulmonary arterial pressure undergoes certain changes during a 3 h period of hypotension (Bø and Hognestad 1971) After the blood loss the pulmonary arterial pressure is thus firstly reduced and reaches its minimum level about 30 min after the start of the bleeding Thereafter this pressure shows a steady increase We decided to examine the fluid volumes of the lung at the time when the pulmonary arterial pressure was at its lowest level *e g* at about 30 min after start of the bleeding In the animals in this group the two lobectomies were therefore carried out with a 30 min interval Hyperinflations could now be performed 15 min before each of the lobectomies One half of these animals was bled between the lobectomies The other half served as control animals

The results from the two groups of animals are shown in Fig 3 In the 5 test cats (mean b w 3 1 kg ± 0 7 S D) with a total blood volume of 159 ml ± 55 S D withdrawal of blood was started immediately after the first lobectomy and continued for about 20—25 min until the femoral arterial pressure was reduced to 50 mm Hg At this time 43 % of the initial total blood volume had been removed. When the second lobe was removed 30 min after the first lobectomy the lung blood content per net dry tissue weight had decreased markedly in all animal with a mean uc

TABLE I Initial values for lung blood content and extravascular water content for 2 groups of cats The values are obtained by removing the right upper lobe after sudden arrest of its circulation All animals were ventilated by positive pressure The animals in group A had been hyperinflated 30 min before the lobectomy The animals in group B had been hyper flated 15 min before the lobectomy Furthermore the animals in the latter group had catheters in the pulmonary artery and in the left atrium as well as an aortic flow probe

	Lung blood content (ml/g dry weight)	Extravascular lung water (ml/g dry weight)
Group A (n = 13)	5 8±1 7 (S D)	4 00±0 23 (S D)
Group B (n = 11)	7 4±1 0 (S D)	3 58±0 28 (S D)
Difference	1 6 (p = 0 004)	0 4? (p = 0 002)

tion of 39 % The extravascular water content increased in 4 and fell in 1 animal with a mean increase of 6 % Pulmonary hematocrit was 68 1 % and 67 5 % of measured peripheral hematocrit in the first and second lobes respectively

Both the ¹Cr and ¹ I concentration in blood decreased in these experiments by % and 21 % respectively The average distribution volume for the isotopes was increased by 16 % after 30 min The vessel hematocrit fell from 35 6 % to 6 %

In 6 control animals (mean b w 3 1 kg ± 0 3 S D) lobectomy no 2 after 30 min showed that the lung blood content per net dry tissue weight had decreased in 5 and increased in 1 with a mean reduction of 10 % Also the extravascular water content was reduced in 5 animals and increased in 1 with a mean reduction of 7 3 % The distribution volume for the isotopes in this group had increased by 7 % without any change in large vessel hematocrit

Again some of the important effects of the blood loss are seen when one compares the second lobes from the bled animals with the second lobes from the control animals (Fig 3) The former lobes showed a significantly reduced lung blood con tent (p = 0 004) as well as a significantly increased extravascular lung water con tent (p = 0 03)

For the evaluation of the method used absolute values for pulmonary blood volume and extravascular water content are of considerable interest These values for all the initially removed lobes (*i e* lobes removed before any changes in total blood volume had been induced) are given in Table I The animals in the group where the effects of a moderate blood loss were tested showed rather uniform values for pulmonary blood volume and extravascular water in the first lobes removed These values were also very uniform within the animal group where effects of a large blood loss were evaluated (Table I However on comparison between the groups mean pulmonary blood volume was considerably higher and mean extra vascular water content considerably lower in the latter group than in the former one (Table I)

The pulmonary hematocrit in the first lobe showed rather uniform values for all the different animals examined The mean value was 69 % (±5 S D) of the measured large vessel hematocrit

In 4 animals the total dry weight of lung tissue was found to be near to 1 g/kg b w (range 0 84—1 4)

Discussion

Sudden arrest of the pulmonary circulation by clamping of the lung hilus and removal of the whole lung has previously been attempted as a method for determination of pulmonary blood volume (Schlant *et al* 1959 Hakkila and Pietila 1961) Only one measurement in each animal could be carried out with the method in this form Since there is such a great interindividual variation in pulmonary blood volume the method has not been found suitable for examination of effects on the pulmonary vasculature of various cardiovascular events

In the present series of experiments one has attempted to avoid this difficulty by removal of two smaller lobes only This makes it possible to evaluate changes in the content of lung blood and of extravascular lung water in the same animal during different circulatory situations Admittedly the method still necessitates tracheostomy and opening of the chest This must necessarily affect the animal s circulatory situation although with practice the surgical procedures can be performed with very little bleeding Still the fluid balance of the animal will be somewhat disturbed at the time when the first lobe is removed

Furthermore the animals were on positive pressure ventilation a condition which will affect both the intra and extravascular fluid compartments These effects of the ventilation were however maintained throughout the experiments and did probably not alter the circulatory adjustments observed in a qualitative way

The blood content and the extravascular water volume found in the first lobe in each animal represent the normal control values of that animal (Table I) Within the same main group the values found in the first lobe were very uniform The difference between the groups as regards lung blood content and lung extravascular water volumes was however unexpectedly large It has been shown that regular hyperinflations will markedly influence the lung blood content and the extravascular lung water content in cats during a period of hemorrhagic hypotension (Aarseth and Bø 1972) The most likely explanation for the differences in these parameters between the two animal groups of the present investigation appears to be the different intervals between hyperinflation and the first lobectomy In the series where the effect of a small blood loss was tested this interval was 30 min In the series with evaluation of a large blood loss the same interval was 15 min only

The total net dry weight of lung tissue was about 1 g/kg b w With about 3 5—4 ml of extravascular water per g dry weight extravascular lung water will thus amount to about 3 5—4 ml/kg b w This is in very good agreement with the values arrived at by Levine Mellins and Fishman (1965) They estimated extravascular

lung water in dogs using a double indicator dilution technique and found a mean value from 41 experiments of 3 5 ml/kg b w ($\pm$0 9 S D) The variation coefficient in their series of experiments was thus 26 % In the present experiments it was 8 % only There is a significant difference between the variation coefficients from the two sets of experiments (p < 0 01 using a F test)

Removal of the upper right lobe caused some immediate changes in arterial pressure as well as in cardiac output (Aarseth and Bø 1972) These parameters stabilized within 5—10 min at values near to what was found before the lobectomy and only small changes in lung blood volume and lung extravascular water content were found in the control animals when the second lobe was removed In the group with 10 min interval between the lobectomies there were a small increase in lung blood content and lung extravascular water volume in the second lobe (Fig 1) In the other control group where the second lobe was removed after 30 min, mean lung blood content as well as lung extravascular water volume had been somewhat reduced (Fig 3) Again the different hyperinflation procedures may explain the difference here between the two control groups In the one group hyperinflation was carried out inbetween the lobectomies whereas this was not the case in the other control group

The uniformity of the obtained values for lung blood content and extravascular water within a group of similarly treated animals indicates that the method is suitable for evaluation of these parameters and of changes in them The markedly different values found in the two control groups do however stress the necessity for careful standardization of the experimental procedure

The effect of a small blood loss on the pulmonary blood content in the cat seemed to be very small (Fig 1) However the actual total blood volume reduction at the time of removal of the second lobe was one third only of the volume of the withdrawn blood The compensatory mechanism of greatest importance here is influx of fluid from the interstitial space (autotransfusion) Some extrusion of erythrocytes from the spleen may also take place The dilution of the intravascular isotopes is largely due to such inward flux of fluid although some fraction of the isotopes will also be lost from the circulation The difference in the degree of intravascular isotope dilution between the animals of the respective control groups on the one hand and the groups of bled animals on the other should reflect the extent of autotransfusion with interstitial fluid and erythrocytes in the latter groups In the moderately bled animal group this difference amounted to about 8 % (10 %—2 %) while for the group with the largest blood loss it was 9 % (16 %—7 %) In the moderately bled animals this calculated autotransfusion before the second lobectomy did thus amount to 2/3 of the induced blood loss It was therefore not surprising that only small actual changes were observed in lung blood content The extravascular lung water content was however significantly reduced (p = 0 033) (Fig 1)

In the other group bled to an arterial mean pressure of 50 mm Hg the calculated autotransfusion amounted to only one fifth of the blood loss The net total blood volume reduction at the time of removal of the second lobe in this group should therefore be about 34 % (43 %—9 %) The pulmonary blood volume of these

animals was found to be reduced by 29 % when compared with the values from the control group (p = 0 004) At the same time the extravascular lung water content increased by 13 % in the bled animals (p = 0 008) (Fig 3)

The various alterations in extravascular lung water content in the different experiments are probably reflections of the direction of capillary hydrostatic pressure changes occurring When the lung water content increased in the markedly bled animals this may thus be caused by a maximal mobilization of blood from the lungs conceivably to a large extent due to postcapillary constriction with increased capillary pressure as its consequence It has thus been shown that the largest increases in extravascular lung water content are to be found in those animals in which the lung blood content was most markedly reduced after the bleeding (Aarseth and Bo 1972)

During moderate hypervolemia in rats it was found that the lung blood volume was almost doubled while the extravascular water content appeared to be somewhat reduced (Aarseth 1971) It was assumed that precapillary constriction with an upstreams vascular dilatation might be the explanation to these findings In the present experiments on cats a moderate hypervolemia resulted in only a moderate increase in lung blood volume Also in these experiments one could however observe some reduction in the extravascular water content When compared to values in the control group this reduction was of the order of 10 % (p = 0 008) (Fig 3) If the same mechanisms are operating in both species then the difference between them as regards lung blood volume increase during hypervolemia may be due to different degrees of distensibility of their pulmonary arterial vessels The pulmonary artery in the rat thus has a relatively poorly developed muscle layer while in the cat this layer is well developed (Fishman 1963) The opened chest and the positive pressure ventilation technique may however also have reduced the blood pooling ability of the cat lungs

With an indicator dilution technique DePasquale Hyman and Burch (1963) and also Kerr and Kirklin (1970) have found large increases in lung blood volume in dogs during induced hypervolemia De Freitas *et al* (1965) and also Korsgren (1966) were however not able to find similar changes in man during hypervolemia. It may be then that different species react differently to hypervolemia buffering it more or less markedly with pulmonary blood volume increases

The mean calculated pulmonary hemaocrit in all the animals of the present experiments was 69 1 % (±5 5 % S D) of the hematocrit measured in blood taken from a larger vessel The same value has been found in rat (Aarseth 1970) In that animal it was observed however that the pulmonary hematocrit changed reciprocally with changes in lung blood volume (Aarseth 1971) It was assumed that varying degrees of laminar plasma flow took place and that the capacitance changes to some extent occurred in small vessels This could have given marked changes in the relative thickness of laminar plasma layers and thus markedly have affected pulmonary hematocrit In the present experiments only small changes in pulmonary hematocrit were found when the lung blood volume changed This could

mean that the more moderate blood volume changes observed in the cat lung does occur in somewhat larger vessels where the relative thickness of the wall near plasma layer would vary less than in smaller vessels

It can be concluded from the present experiments that also the pulmonary vascular bed of the cat is an efficient blood depot from where blood can be expelled during a blood loss The pulmonary blood volume changes observed were somewhat less marked than those found in rats The findings that extravascular lung water volume was reduced both during moderate hypovolemia and during hypervolemia are in accordance with results obtained in rats (Aarseth 1970 1971) However, during hypervolemia the pulmonary vascular bed in cats was distended much less than was the pulmonary vasculature in rats This may be due to a species difference and/or to the different techniques employed

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Pulmonary Blood- and Extravascular Water Volumes during Prolonged Post Hemorrhagic Systemic Hypotension

By

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Abstract

Aarseth P and G Bo *Pulmonary blood and extravascular water volumes during prolonged post hemorrhagic systemic hypotension* Acta physiol scand 1972 *85* 353—361

In cats prolonged hemorrhagic hypotension had been shown to increase the pulmonary arterial pressure and vascular resistance Pulmonary extravascular water volume and pulmonary blood volume have been evaluated early and late in such a period of post hemorrhagic hypotension. ^{51}Cr labelled erythrocytes and ^{12} I labelled albumin were injected intravascularly to animals with an opened chest and on positive pressure ventilation One upper lung lobe was then suddenly clamped removed and frozen The lung lobe tissue and a simultaneously taken blood sample from a large vessel were measured for total weight dry weight and tracer content The lobes blood volume and extravascular water volume were then calculated per unit extravascular lung dry weight A second lung lobe was removed and treated similarly at a later stage 1/2 h after the blood loss there was a marked reduction in lung blood volume and at the same time some increase in pulmonary extravascular water volume indicating constriction of postcapillary capacitance vessels After 3 h of hypotension pulmonary extravascular water volume was found to be the same as before the hemorrhage although the pulmonary arterial pressure increased This indicated that the increase in vascular res stance must predominantly be due to vasoconstriction at precapillary sites The reduction in the lungs blood volume remained at these late stages indicating that capacitance vessels were still constricted When hyperinflations of the lungs were omitted in the experimental period there was a tendency towards water accumulation in the lungs

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In dogs (Cook and Webb 1968) as well as in cats (Bo and Hognestad 1971) pulmonary arterial hypertension has been shown to develop during a period of post hemorrhagic hypotension The precise mechanisms behind this pulmonary hypertension is still not clear Histological examination of the lungs of such animals have revealed congestive atelectasis hemorrhagic patches and edema especially in the basal regions of the lungs (Henry *et al* 1967 Cook and Webb 1968) It has also been shown that histological changes of this type can be diminished or even prevented by regularly performed hyperinflations of the lungs during the hypotensive period (MacKay *et al* 1969) In the investigations of Bo and Hognestad (1971) on cats regular hyperinflations were carried out t hout the experiments This m

the reason why in these experiments there was no histologic evidence for the presence of pulmonary edema after the period of hemorrhagic hypotension Still a marked pulmonary hypertension did develop also in these animals The findings in these animals are thus apparently at variance with the proposal that post capillary vasoconstriction in the lung with increased capillary pressure and edema formation plays an important part in the development of the post hemorrhagic pulmonary hypertension (Cook and Webb 1968)

In the present experiments cats with open chests and on artificial ventilation have been exposed to a hemorrhage with a subsequent hypotensive period During this period changes in extravascular water volume and in the blood volume of the lungs have been evaluated For this purpose suitable tracers were introduced intravascularly and their concentration later measured in extirpated lung lobes The intention was to gain information about where in the pulmonary vasculature the increase in resistance did actually take place At a time when the pulmonary arterial pressure was markedly increased the lung blood volume was found to be reduced whereas the pulmonary extravascular water content showed only very small changes

Methods

Animals Cats weighing from 2 6 to 4 6 kg were anesthetized by i p injections of sodium pentobarbitone (Nembutal® Abbott) 30—40 mg/kg

Ventilation After tracheostomy a muscle relaxant Alloferin® Roche 0 5 mg/kg was given and positive pressure ventilation started (The Ideal Respiration Pump C F Palmer Ltd London) The respiratory frequency was 21 per min By the use of water seals the end tidal pressures were kept constant at 4 to 6 and 1 cm H O respectively Hyperinflations at a transpulmonary pressure of 15 cm H_2O were carried out twice per h and 15 to 20 min before the lobectomies In 4 animals hyperinflations were omitted after the first lobectomy The ventilation was adjusted so that the arterial pH was kept between 7 35 and 7 45 in the normotensive control animals and up to the point of bleeding in the others The pH measurements were carried out with a Radiometer pH meter 22 equipped with the electrode G 297/G

Hematocrit values were determined by the use of an International Microcapillary centrifuge Model MD

Preparation The chest was opened widely by splitting the sternum Polyethylene catheters were introduced into the pulmonary artery and the left atrium for recording the pulmonary arterial pressure P_{PA} and the left atrial pressure P_{LA} respectively Statham pressure transducers P23 Gb and P23 De were used for these measurements Catheters were also introduced *into both femoral arteries for recording the femoral blood pressure* P_{FA} *(with* a Statham P23 Db transducer) and for blood sampling The cardiac output was measured by a Nycotron square wave electromagnetic flowmeter (type 372 Nycotron A/S Norway) using a wraparound flowprobe on the ascending aorta When the surgical procedures had been finished the animal was left undisturbed for 20—30 min in order to obtain stable values for cardiovascular pressures and for cardiac output All the parameters were recorded on a six channel Sanborn recorder (Model 320 Sanborn Co California) The body temperature was maintained by leading warm moist air into a transparent polyethylene tent covering the animal

Bleeding procedure Heparin 500 I U /kg was given i v (Heparin AL®) The animals were then bled through the femoral artery until the femoral arterial pressure had fallen to 50 mm Hg By further small bleedings or retransfusions the systemic blood pressure was then kept at that level

The lobectomies After the splitting of the sternum loops of silk threads were placed loosely around the hilus of both upper lobes and in some experiments also around the hilus of the right middle lobe Lobe blood flow and ventilation was arrested at the end of an expiration by suddenly tightening the silk ligature around the hilus A special designed instrument a tightener was used for this procedure and for keeping the hilus clamped while a ligature was put around it Special care was taken to arrest the lobe s blood flow as quickly as possible without disturbing the circulation through the rest of the lung The isolated lobe was then

removed and frozen in liquid nitrogen Because of its stalklike hilus the right upper lobe was extirpated first The blood flow to the lower lobes of the right lung seemed undisturbed by the removal of the upper lobe

The radioisotopes Human serum albumin tagged with ^{125}I and the animal s own erythrocytes tagged with ^{51}Cr (isotopes delivered by Institutt for Atomenergi Kjeller Norway) were used for measurements of the plasma and erythrocyte volumes The tracer material was injected i v 10 min before the first lobectomy and the first blood sampling were carried out In 8 expts in which the two lobectomies were spaced by 3 h labelled erythrocytes only were given initially Labelled albumin was then given 10 min before the second lobectomy

Calculations In principle the erythrocyte volume of the lobe was found by division of the lobe s total ^{51}Cr content with the ^{51}Cr content in a large vessel blood sample of known weight and hematocrit Similarly the plasma volume in the lobe was found by division of the ^{125}I content of the lobe with the ^{125}I content in a large vessel plasma sample of known volume

Since the labelled albumin is continuously lost from the blood into the tissues the plasma ^{125}I content reflects plasma volume only for a limited period of time Therefore the animals used in the 3 h expts received only labelled erythrocytes before the first lobectomy However pulmonary hematocrit and total body hematocrit has previously been found to be remarkably uniform in a large group of animals (Aarseth and Bo 1972) These hematocrit values which were 69 and 95 per cent of large vessel hematocrit respectively could be used for calculations of both lung lobe blood volume and total blood volume The basis of these calculations were the measured lobar erythrocyte volumes and the measured large vessel *hematocrit in each individual animal When then labelled albumin was given late in the course* of the experiments on these animals it was confirmed that their pulmonary hematocrit was really the same as that found in other animals namely 68 1 per cent of large vessel hematocrit

The extravascular water volume of a lobe was obtained by subtracting the wet weight of the lobe s plasma and erythocyte volumes from the total wet weight of the lobe The net dry weight of a lobe was found by subtracting the dry weight of the lobe s plasma and erythrocyte volume from the total dry weight of the lobe Blood content and extravascular water volume in a lobe were expressed as ml/g net dry weight of lobe

In the results extravascular water volumes and blood contents of the lungs are given as relative values only Thus the values from lobes 2 and 3 are given as per cent of the values in lobe 1

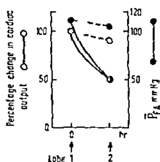
More detail about the preparation and use of the isotopes and the calculations employed are given in a preceding paper (Aarseth and Bo 1972)

Results

Control animals The circulatory effects of a lobectomy were studied in 6 non bled animals In this control group the second lobectomy was performed 30 min after the first one The performance of a lobectomy was found to cause a transient rise in pulmonary arterial pressure and some fall in cardiac output Mechanical traction on the hilus did probably play a role for these changes Within a few min after the lobectomy pulmonary arterial pressure and cardiac output stabilized at levels near the initial values (Fig 1) Comparisons between the first and the second lobes showed that the extravascular water volume and the blood content of the second lobes had fallen by 7 3 and 9 7 per cent respectively (Table I)

Bled animals The pressure flow relationship in the lungs of these animals changed with time as described earlier (Bo and Hognestad 1971) About 30 min after the induced systemic hypotension the P_{PA} reached its lowest level which was about 2—4 mm Hg below the initial value The cardiac output was then reduced by about 50 per cent (Fig 1) A gradual rise in P_{PA} then occurred and after 3 h it was 50 per cent higher than initially whereas cardiac output still remained low

Three groups of bled animals were used for the examination of lung blood content and extravascular water volume during this development of pulmonary arterial



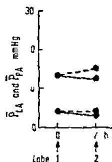


Fig 1 Changes in cardiac output mean femoral arterial pressure P_{FA} mean pulmonary arterial pressure P_{PA} and mean left atrial pressure P_{LA} in two groups of cats The values given are based on measurements just before a first and a second lobectomy Anesthetized cats with open chests and on positive pressure ventilation were used (see Methods) The broken line represents mean values in a control group of 6 non bled animals The solid line represents mean values from a group of 5 animals exposed to a large hemorrhage (see Methods) Bleeding was carried out after the first lobectomy (see text)

hypertension The effect of regular hyperinflations on the fluid content in the lungs of such animals (MacKay *et al* 1969) was also examined In all animals the right upper lobe was removed before the bleeding was started In one group of 5 animals the second lobe was removed 30 min after the blood loss In a second group of 4 animals hemorrhagic hypotension was maintained during a 3 h period before the second lobectomy was carried out In this interval hyperinflations were performed regularly A third group of 4 animals was also kept hypotensive during 3 h but here the regular hyperinflations were omitted after the first lobectomy

The results from the first group are shown in Fig 1 and Table I The extravascular fluid volume in the second lobes removed had risen by 6 per cent whereas the pulmonary blood volume had fallen by 39 per cent

In Fig 2 and Table I and II are shown the results from the animals which were kept hypotensive during 3 h before their second lobe was removed In the animals which had received regular hyperinflations during the hypotensive period there was

TABLE I Changes in extravascular pulmonary water volume and in pulmonary blood content in open-chested cats subjected either to no bleeding at all to a short lasting post hemorrhagic hypotension or to a more long lasting hypotension Changes evaluated by comparison between a first lobe removed initially and a second one removed at a later stage Changes given as per cent of values in first lobe

	Second lobe removed after 1/2h No bleeding (control animals) (n 6)	Second lobe removed after 1/2h of hemorrhagic hypotension (n=5)	Second lobe removed after 3 h of hemorrhagic hypotension Regular hyperinflations carried out (n = 4)
Percentage changes in extra vascular pulmonary water volume	−7 3 ° S E = ±4 5	+6 ° S E = ±4 3	0 S E = ±4 8
Percentage changes in pulmonary blood content	−9 7 S E = ±5 9	−39 S E = ±5 1	−20 S E = ±6 1

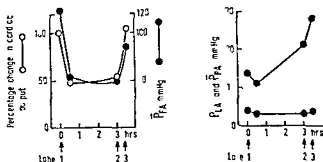


Fig 2 Changes in cardiac output and in femoral arterial (P_{FA}) pumonary arterial (P_{PA}) and left atrial (P_{LA}) pressures during a 3 h period of posthemorrhagic systemic hypotension. The values given are based on measurements carried out just before a first a second and a third lobectomy (see Methods) Mean values for 8 bled animals are given 4 of them had had regular hyperinflations and 4 had had no such hyperinflations during the 3 h hypotensive period There were no systematic difference between the results from the two groups of animals The results are therefore combined

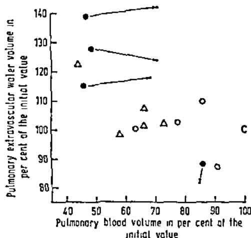
no change in the extravascular pulmonary water volume from the first to the second lobe The blood volume of the second lobe was however 20 per cent below the value in the first lobe

In the animals which had had no hyperinflations during the hypotensive period the extravascular water volume increased by 39 28 and 15 per cent in 3 animals and decreased by 12 per cent in the fourth The mean change was a 17 5 per cent increase (Table II) The pulmonary blood volume in the second lobe of this group was markedly reduced the mean value being 43 per cent of the average value in the first lobes

After the second lobectomy each of 8 hypotensive animals were retransfused with their own blood Their main total blood volume after the retransfusions was found to be 113 per cent of the initial values The cardiac output was increased towards its initial value whereas the P_{PA} showed a further increase (Fig 2) The systemic blood pressure did however remain below its initial level thus indicating a persistent decrease in total peripheral vascular resistance

TABLE II Changes in extravascular pulmonary water volume and in pulmonary blood content in open-chested cats subjected to long lasting hemorrhagic hypotension Hyperinflations omitted during the hypotensive period Changes evaluated by comparison between a first lobe removed initially a second lobe removed after 3 h and a third lobe removed after retransfusion of the animal Changes given as per cent of values in first lobe

Number of animals 4	Second lobe removed after 3 h of hemorrhagic hypotension	Third lobe removed after 3 h of hemorrhagic hypotension and subsequent retransfusion
Percentage changes in extravascular pulmonary water volume	+17 5 S E = ±11	+16 S E = ±11
Percentage changes in pulmonary blood content	−43 S E = ±10	−26 7 S E = ±4



△ Values from 5 bled animals in which the second lobe was taken after 30 min

○ Values from 4 bled animals in which the second lobe was taken after 3 h of hypotension Hyperinflations had been carried out twice per h during the hemorrhagic period

● Values from 4 bled animals in which the second lobe was taken after 3 h of hypotension and where hyperinflations of the lungs were omitted during the hemorrhagic period

→ The arrows are pointing towards the values in a third lobe from the last group of animals This lobe was removed after retransfusion of the animals with their own shed blood

Fig 3 Changes in pulmonary extravascular water volume and pulmonary blood volume in a second excised lobe from three groups of bled animals The C mark represents the initial values of the first (control) lobe which was set to 100 per cent.

In this situation with an increased total blood volume a normal cardiac output but a markedly increased P_{PA} a third lobe the right middle lobe was removed from animals where no hyperinflations had been carried out The pulmonary blood volume was found to be increased when compared to the value found in the second lobe but was still 27 per cent below the pre bleeding value (Table II) The extravascular water content underwent no change from the second to the third lobe in these 4 animals (Table II and Fig 3)

Discussion

The main purpose of this work has been to obtain information about the extravascular fluid content in lungs where a pulmonary arterial hypertension had developed during a period of post hemorrhagic systemic hypotension Changes in the extravascular water volume during hemorrhage have been described and followed in the cat's hind part (Mellander and Lewis 1963) We assumed that we should be able to detect and interpret changes of this type also with the chosen technique in the present lung preparation

A lobectomy will by itself cause some reduction of the pulmonary vascular bed and could also induce vascular reflexes The effects of the lobectomy on pulmonary arterial and systemic blood pressures and on cardiac output were however small and transient (Fig 1) Moreover in control animals the blood content and the extravascular water volume in a second lobe removed after 30 min were not much changed from the values in a first lobe This indicates that the method of comparing the conditions in two separately removed upper lobes is reliable and useful for our purpose

The immediate effects of hemorrhage on the systemic vascular beds are vasoconstriction of capacitance vessels and increase in precapillary resistance with fall in capillary pressure and influx of water into the vascular bed However when the systemic hypotension in the cat is prolonged for more than 1 h the precapillary vasoconstriction gradually decreases Since the constriction of postcapillary vessels persists the capillary pressure will now increase and efflux of water takes place (Mellander and Lewis 1963) In the present type of experiments the reduced total peripheral resistance after 3 h (Fig 2) and the need for small repeated retransfusions in order to maintain a stable systemic arterial pressure indicate that such a gradual relaxation of systemic precapillary vessels had occurred

In the lobes removed after 30 min of hypotension the extravascular water volume had increased by 6 per cent (Table I) When compared to the condition in the second lobes of the non bled control animals (Table I) this was a significant increase ($P < 0.05$) An increase in capillary hydrostatic pressure has thus apparently taken place although the P_{PA} was lowered (Fig 1) At the same time a marked reduction in lobe blood volume had taken place (Table I) It is tempting to conclude that some constriction of postcapillary vessels must have taken place causing a capillary pressure rise and the capacitance reduction

In lobes which had been regularly hyperinflated and which were removed after 3 h of hemorrhagic hypotension no change in extravascular water was found (Table I) The pulmonary capillary pressure has thus probably been near its pre bleeding value despite the increase in pulmonary arterial pressure (Fig 2) The slowly developing increase in pulmonary vascular resistance in these animals must therefore apparently mainly be located to precapillary vessels

The pulmonary vascular reaction picture subsequent to a bleeding is thus apparently different from what is seen in the vascular bed of skeletal muscle This is not surprising in view of the fact that the nutritive role and the transmural pressures are completely different for lung and skeletal muscle vasculature

The non hyperinflated bled animals showed the same increase in the P_{PA} as did the others In 3 of these animals the extravascular water volume did however show an increase This indicates that a more pronounced increase in postcapillary resistance takes place when hyperinflations are omitted

When these animals were retransfused with their shed blood and a third lobe removed no further increases in extravascular water volume were found This and the above finding indicate that the cat possesses effective mechanisms for protection of the pulmonary capillaries against pressure rises from the pulmonary arterial side In the dog it has been shown that mechanical distension of the great pulmonary arteries induces pulmonary precapillary constriction (Hyman 1968) A similar mechanism may exist also in the cat The decrease in pulmonary extravascular water volume following induced hypervolemia in the cat may also be the result of protective precapillary constriction (Aarseth and Bø 1971)

The magnitude of net water efflux across the pulmonary capillaries upon a elevation of the pulmonary capillary pressure is not exactly known In a-

and perfused lung preparation an increased, but stable weight of the preparation could be obtained after elevations of the capillary pressure by 5—10 mm Hg (Lunde and Waaler 1969) The amount of fluid which had then been filtered out of the vessels was difficult to evaluate exactly in that preparation but it was assumed to be of the order of 0 5 g or 7—10 per cent of the extravascular water volume The moderate increase in extravascular water volume found in our experiments could thus have been caused by appreciable elevations of capillary pressure According to Levine Mellins and Fishman (1965) extravascular water volume has to be increased by as much as 70 per cent before pulmonary edema is apparent Even the most marked increase in extravascular water volume found in the present experiments (39 per cent) was therefore considerably below such a limit for evident pulmonary edema This lack of edema after a 3 h hypotensive period is in agreement with the observations of Bø and Hognestad (1971) Also the conclusions of MacKay *et al* (1969) that regular hyperinflations prevent fluid accumulation in the lungs during hemorrhagic hypotension is supported by our findings Our results allow no explanation for this protective action of hyperinflations

The pulmonary blood volume is large when seen in relationship to the weight of the organ Aarseth (1970) has shown that the pulmonary blood content represents a blood depot which may be drawn upon after acute hemorrhage This finding is confirmed in the present experiments The pulmonary blood volume is apparently to extent influenced by the level of P_{PA} The pulmonary blood volume did thus rease in the period where the P_{PA} rose markedly (Table I and Fig 2) In the non hyperinflated animals the pulmonary blood volume did also increase subsequent to the retransfusion where at the same time the P_{PA} also increased (Table II and Fig 2) These findings seem together with the unchanged extravascular water volume to indicate distension of pulmonary arterial vessels upstreams to the site of some precapillary vasoconstriction

It is interesting that the pulmonary blood volume was found to be reduced also in the retransfused hypervolemic animals with normal cardiac output and increased P_{PA} (Fig 2 and Table II) This indicates that active vasoconstriction still prevails in precapillary resistance vessels as well as in vessels with capacitance function The lowest values of the pulmonary blood volume seen 50 per cent reduction from the initial level were found in animals which had a 15 to 39 per cent increase in the extravascular water volume (Fig 3) Small reductions in pulmonary blood volume were found in animals which had no changes in extravascular water volume This is another indication of vasoconstriction in postcapillary capacitance vessels being involved at least during very marked pulmonary blood volume reductions

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Gastric Absorption of L(+) and D(−) Lactic Acid and their Effects on the Transmucosal Ion Transport in Innervated, Non secreting Cat Stomachs

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Abstract

FRENNING B *Gastric absorption of L(+) and D(−) lactic acid and their effects on the transmucosal ion transport in innervated non secreting cat stomachs* Acta physiol scand 1972 *85* 362—373

instillation of 170 mM lactic acid (L or D) into unstimulated whole stomach pouches in cats the hydrogen ion concentration decreased more slowly than on instillation of 170 mM hydrochloric acid When 700 mM lactic acid (L or D) was instilled the reverse was found On instillation of 170 mM HCl subsequent to an instillation of 170 mM lactic acid (L or D) the changes in concentration of electrolytes as well as the net fluxes of electrolytes were of the same order of magnitude as during a preceding control instillation of HCl On instillation of 170 mM HCl subsequent to an instillation of 700 mM lactic acid (L or D) the decrease in hydrogen ion concentration and the increase in sodium ion concentration were significantly larger than in the control instillation of HCl The net influx of sodium ions was significantly increased but not the net efflux of hydrogen ions After exposure of the gastric mucosa to 700 mM lactic acid (L or D) it produced a mucoid fluid that contained mainly sodium and chloride ions This explained the results obtained when HCl was instilled subsequent to an instillation of 700 mM lactic acid No morphological changes were found on examination in the scanning electron microscope of gastric mucosae exposed to 6 ml of 700 mM L lactic acid

On instillation of relatively small amounts (5—10 ml) of 170 mM hydrochloric acid into tied-off unstimulated cat stomachs the hydrogen and chloride ion concentrations in the instillate decrease and the sodium and potassium ion concentrations increase usually without any appreciable net movement of fluid This was shown by Teorell (1933 1939) and has been repeatedly confirmed Expts on cats with innervated whole stomach pouches have shown that exposure of the mucosa to acetic or acetylsalicylic acid increases its permeability to ions (Hemstrom Frenning and Öbrink 1964 Hemstrom and Frenning 1968 Frenning 1971) A different view is held by Davenport (1964 1967 and 1970) who from the result of instillations of relatively large amounts (30 ml) of acid test solutions into denervated (Heidenhain) gastric pouches in dogs claims that the normal mucosa offers barriers against trans

mucosal transport of electrolytes. Subsequent to repeated instillations of relatively large amounts of acetic, propionic, butyric, acetylsalicylic or salicylic acid he obtained large net losses of hydrogen ions and large net gains of sodium ions on instillation of the acid test solution, an effect attributed to "breaking of the barrier". Differences in experimental techniques and in nomenclature probably account for the different interpretations of the effect of these acids.

A permeability increasing effect of acetic or propionic acid has been shown also on the isolated frog gastric mucosa and evidence supporting transient intracellular accumulation of acid, presumably in an ionized form after exposure of the secretory side of the mucosa to acetic, propionic or lactic acid (pH = 4.00) has been presented (Flemström 1971). Frenning and Öbrink (1971) examined in the scanning electron microscope cat gastric mucosae exposed to acetic or acetylsalicylic acid and found that the surface epithelial cells, in contrast to what was found in normal stomachs, were swollen and that the intercellular junctions (see Farquhar and Palade 1963) appeared to be partially severed. Against this background it was considered of interest to determine whether L(+) or D(−) lactic acid (α-hydroxypropionic acid) also influenced the gastric mucosal permeability for ions and whether exposure to lactic acid changed the morphology of the gastric mucosa as observed in the scanning electron microscope.

Methods

Experimental animals

The expts. were performed on cats (mean wt 3.0 kg, range 2.2—4.4 kg, n = 25) which had been starved for at least 18 h but given free access to water. Anesthesia was induced with Fluothane® (Halothane®) or chloroform and maintained with chloralose (70 mg/kg b.wt.) and urethane (0.2—0.6 g), both given i.v. The stomach was isolated by ligatures at the cardia and the pylorus, care being taken not to disturb the gastric blood and nerve supplies. A glass cannula was inserted in the pyloric end of the stomach and the abdomen was closed. The stomach was then rinsed with physiological saline. Before starting the expts. there was a resting period of 2—3 h, during which the secretory condition of the stomach was checked. The body temperature was 38.1±0.2 °C at the start of the expts. and 38.2±0.2 °C at the end (mean ±S.E., n = 22). The mean blood pressure was initially 143±6 mm Hg and at the end of the expts. 128±5 (n = 21).

Analysis

Acidity determination. 0.05 ml samples were diluted in 5 ml distilled water and titrated with 10 mM NaOH (indicator bromthymol blue).

Chloride was determined electrometrically on the same samples as were used for acidity determination (Auto Burette Unit, type ABU12, pH Meter Type PHM 26c, Radiometer, Copenhagen, Denmark). 5 mM $AgNO_3$ was used for titration.

Sodium and potassium were determined flame photometrically after apropriate dilution with distilled water (flame photometer Eppendorf, Netheler and Hinz GMBH, Hamburg).

L(+) lactate was determined enzymatically according to Scholtz, Schmitz, Bucher and Lampen (1959). The reagents were obtained from Boehringer & Soehne GMBH, Ingelheim, Germany. *D(−) lactate* was not determined.

The coefficient of variation for sodium determination was ±2 % at 100 mM. For the acidity, chloride and lactate determinations the coefficients of variation were smaller. (The coefficients were determined from analysis of 10 samples from the same solutions.)

Chemicals

The D(−) lactic acid used contained 3 % L(+) lactic acid. The L(+) lactic acid used was of highest available purity (98—9[illegible]). Both were obtained form Sigma Chemical Co., St. Louis, Mo., USA.

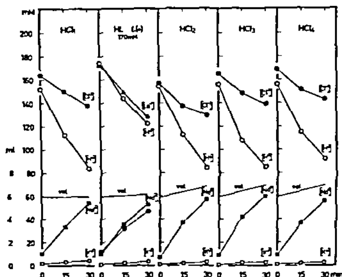


Fig 1 The result of an expt. in which 6 ml of 170 mM HCl was instilled into a tied-off nonsecreting cat stomach once before and repeatedly after an instillation of 6 ml of 170 mM L(+) lactic acid

Experimental procedures

The electrolyte output in the last 30 min of the resting period (the basal electrolyte output) was determined The expts were then begun with a 30 min instillation of 6 ml of 170 mM HCl This period is referred to as HCl_1 or control period. 6 ml of 170 or of 700 mM L(−) D(−) lactic acid was then instilled for an equal length of time This was followed by 2 or 3 consecutive 30 min instillations of 170 mM HCl referred to as HCl HCl_3 and HCl_4 (As a 4th HCl instillation was not performed in all of the expts and the results of those that were performed did not give any further information they are not reported in detail) Initial samples were taken within 1 min At 15 min samples were taken without complete draining of the stomach At 30 min the stomach was drained as completely as possible the volume measured to the nearest 0 1 ml in a fine-graded measuring cylinder and samples taken On each occasion a total of 0 1 ml was used for determination of hydrogen chloride sodium and potassium A further 0 05 ml was used for L(+) lactate determination. Before HCl was instilled subsequent to instillation of 700 mM lactic acid the stomach was rinsed with HCl otherwise no rinsing was performed between the different instillation periods The changes in concentration of hydrogen, sodium potassium, chloride and lactate given comprise the differences in concentration between the final and the initial samples. The reported changes in volume of the instilled solutions are corrected for the volume of the samples taken The net fluxes of ions reported comprise the differences between recovered and instilled amounts

Scanning electron microscopy

Specimens from 3 stomachs fixed immediately after a 30 min instillation of 700 mM L(+) lactic acid and from untreated stomachs were examined in a Jeol scanning electron microscope (JSM U3) operated at 15 kV For description of the preparatory procedure used see Frenning and Öbrink (1971)

Results

Absorption of lactic acid

On instillation of 170 or 700 mM L(+) lactic acid the decreases in hydrogen ion and lactate concentration were essentially equal (Fig 1 and 3 Table I) The net effluxes of hydrogen ions and lactate were also roughly equal (see Table II) Note the different ways in which the changes in concentration and the net fluxes of ions were determined No determinations of D(−) lactate were performed but as the decrease in hydrogen ion concentration and the increases in sodium potassium and

TABLE I Changes in concentration of electrolytes and in volume on 30 min installations of 6 ml of 170 mM hydrochloric acid or of lactic acid into unstimulated whole gastric pouches in cats HCl is the control period HCl and HCl were performed 0–30 and 30–60 min respectively after removal of the lactic acid The values given are mean ± S E

Experimental period	n	ΔH+ (mM)	ΔCl− (mM)	ΔNa+ (mM)	ΔK+ (mM)	ΔLactate (mM)	ΔV (ml)
HCl		−65±4	−20±3	+41±2	+3±1		+0 3±0 4
170 mM L(+) lactic acid	5	−52±1	+48±4	+43±1	+4±0	− 56±5	+0 5±0 3
HCl		−75±3	−29±1	+50±2	+4±1		+0 5±0 5
HCl		−71±1	−25±3	+49±2	+4±1		+0 8±0 6
HCl		−67±6	−21±3	+46±4	+3±0		+0 9±0 2
700 mM L(+) lactic acid	6	−292±8	+51±3	+61±2	+5±0	−287±8	+2 1±0 4
HCl		−100±4	−30±1	+70±3	+4±0		+3 0±0 4
HCl		− 89±6	−25±2	+64±2	+3±0		+2 7±0 4
HCl		−68±6	−19±4	+47±3	+3±0		+0 4±0 3
170 mM D(−) lactic acid	3	−54±3	+49±1	+46±1	+3±0		+0 6±0 4
HCl		−73±6	−29±5	+48±3	+3±0		+1 0±0 1
HCl		−71±4	−21±1	+51±2	+3±0		+1 2±0 2
HCl		−60±5	−18±2	+41±2	+3±0		+0 3±0 ?
700 mM D(−) lact c acid	3	−29? ±15	+49±3	+64±4	+5±1		+1 4±0 1
HCl		− 97±12	−?8±1	+70±3	+3±0		+2 2±0 6
HCl		− 81±1	−22±1	+61±4	+3±0		+3 0±0 3

TABLE II The mean net fluxes of electrolytes ± S E in the same expts as presented in Table I The mean basal electolyte output in 30 m n preceding the start of the expts is also given

Experimental period	n	H+ net (μEq/30 m n)	Cl net (μEq/30 m n)	Na+ net (μEq/30 min)	K+ net (μEq/30 min)	Lactate net (μEq/30 min)	ΔV (ml)
Basal output		+11 +8	+83 ±4	+65 ±5	+7 ±1		+0 4±0 1
HCl		−436 ±36	−77 ±66	+348 ±33	+33 ±4		+0 3±0 4
170 mM L(+) lact c ac d	5	−310 ±34	+387 ±43	+326 ±?2	+28 ±3	−333±17	+0 5±0 3
HCl		−446 ±40	−205 ±48	+368 ±36	+31 ±5		+0 5±0 5
HCl		−420 ±42	−46 ±87	+394 ±53	+32 ±6		+0 8±0 6
Basal output		+11 ±4	+76 ±15	+62 ±10	+4 ±1		+0 5±0 1
HCl		−374 ±49	+16 ±47	+408 ±37	+28 ±3		+0 9±0 2
700 mM L(+) lact c acid	6	−1691 ±206	+524 ±52	+495 ±39	+42 ±5	−1789±251	+2 1±0 4
HCl		−359 ±40	+195 ±72	+704 ±41	+4? ±4		+3 0±0 4
HCl		−412 ±28	+233 ±63	+697 ±55	+34 ±2		+2 7±0 4
Ba al output		+6 ±2	+45 ±16	+35 ±13	+2 ±1		+0 3±0 1
HCl		−456 ±48	−39 ±47	+361 ±?1	+24 ±1		+0 4±0 3
170 mM D(−) lactic acid	3	−336 ±33	+401 ±49	+351 ±34	+22 ±1		+0 6±0 4
HCl		−399 ±1	−74 ±17	+381 ±22	+22 ±1		+1 0±0 1
HCl		−395 ±21	+6 ±23	+402 ±15	+23 ±1		+1 2±0 2
Basal output		+19 ±7	+111 ±27	+86 ± 0	+8 ±.		−0 7±0 2
HCl		−406 ±41	−67 ±40	+331 ±10	+26 ±3		+0 3±0 2
700 mM D(−) lact c ac d	3	−1757 ±238	+431 ±33	+485 ±33	+39 ±5		+1 4±0 1
HCl		−367 ±34	+39 ±74	+658 ±63	+36 ±6		+2 2±0 6
HCl		−382 ±19	+250 ±28	+699 ±81	+39 ±4		+3 0±0 3

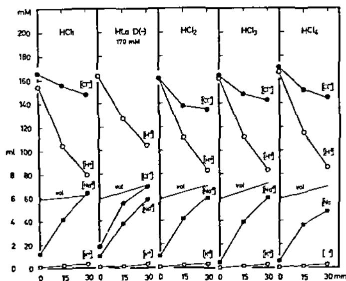


Fig 2 An expt similar to that shown in Fig 1 the only difference being that the D(−) isomer of lactic acid was used in the intervening instillation

chloride ion concentration were of the same order of magnitude on instillation of 170 or 700 mM D(−) lactic acid as on instillation of 170 or 700 mM L(+) lactic acid respectively the absorption of D(−) lactate probably also equalled the absorption of L(+) lactate The decrease in hydrogen ion concentration was smaller on in on of 170 mM lactic acid than on instillation of 170 mM HCl (Fig 1 and 2 Table I) When 700 mM lactic acid was instilled i e when the lumen to blood concentration difference was considerably increased the reverse was found Neither during instillation of 170 mM L(+) nor of 170 mM D(−) lactic acid did any decrease in volume occur though the solutions were hypoosmotic 30 min instillations of 700 mM L(+) or D(−) lactic acid resulted in mean volume increases which exceeded those in the corresponding control periods by 1 2 and 1 1 ml respectively

170 mM lactic acid and gastric transmucosal ion transport

On instillation of 170 mM HCl subsequent to an instillation of 170 mM L(+) lactic acid the decreases in hydrogen and chloride ion concentration and the combined increase in sodium and potassium ion concentration were of the same magnitude as in the control period The same was found in the following HCl instillations (see Fig 1 and Table I) The net effluxes (from the gastric lumen) of hydrogen and chloride ions and the net influxes (into the gastric lumen) of sodium and potassium ions on instillation of HCl were also unchanged after exposure of the mucosa to 170 mM L(+) lactic acid (see Table II) There was essentially no difference when the stomach was exposed to the same concentration of the D(−) isomer (see Fig 2 and Table I and II) Thus an instillation of 170 mM lactic acid (L or D) did not in any respect influence the net gastric transmucosal ion transport on subsequent instillation of hydrochloric acid

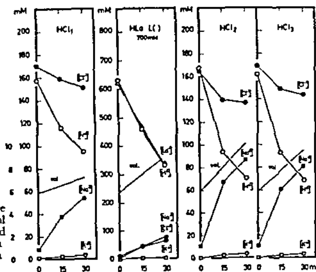


Fig 3 An expt. showing the effect of an instillation of 6 ml of 700 mM L(+) lactic acid on the changes in concentration of electrolytes on instillation of 170 mM HCl

700 mM lactic acid and gastric transmucosal ion transport

The results are presented in Fig 3—5 and Table I II and III On instillation of 170 mM HCl subsequent to an instillation of 700 mM L(+) lactic acid (Fig 3 Table I) the decreases in hydrogen and chloride ion concentration and the increase in sodium ion concentration were significantly larger than in the control instillation of HCl ($p < 0.01$, 0.05 and 0.02 respectively) The increase in volume was significantly larger than in the control period ($p < 0.01$) During HCl_3 the decrease in hydrogen ion concentration and the increase in sodium ion concentration and in

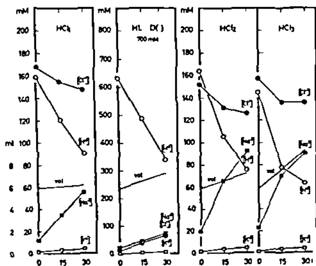


Fig 4 An expt. similar to that shown in Fig 3 the only difference being that the D(−) isomer of lactic acid was used in the intervening instillation.

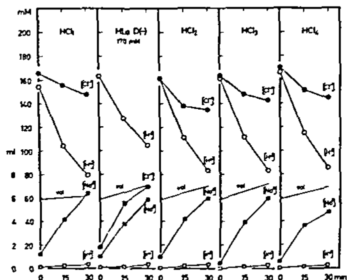


Fig 2 An expt. similar to that shown in Fig 1 the only difference being that the D(−) isomer of lactic acid was used in the intervening instillation

chloride ion concentration were of the same order of magnitude on instillation of 170 or 700 mM D(−) lactic acid as on instillation of 170 or 700 mM L(+) lactic acid respectively the absorption of D(−) lactate probably also equalled the absorption of L(+) lactate The decrease in hydrogen ion concentration was smaller on instillation of 170 mM lactic acid than on instillation of 170 mM HCl (Fig 1 and 2 Table I) When 700 mM lactic acid was instilled i e when the lumen to blood concentration difference was considerably increased the reverse was found Neither during instillation of 170 mM L(+) nor of 170 mM D(−) lactic acid did any decrease in volume occur though the solutions were hypoosmotic 30 min instillations of 700 mM L(+) or D(−) lactic acid resulted in mean volume increases which exceeded those in the corresponding control periods by 1 2 and 1 1 ml, respectively

170 mM lactic acid and gastric transmucosal ion transport

On instillation of 170 mM HCl subsequent to an instillation of 170 mM L(+) lactic acid the decreases in hydrogen and chloride ion concentration and the combined increase in sodium and potassium ion concentration were of the same magnitude as in the control period The same was found in the following HCl instillations (see Fig 1 and Table I) The net effluxes (from the gastric lumen) of hydrogen and chloride ions and the net influxes (into the gastric lumen) of sodium and potassium ions on instillation of HCl were also unchanged after exposure of the mucosa to 170 mM L(+) lactic acid (see Table II) There was essentially no difference when the stomach was exposed to the same concentration of the D(−) isomer (see Fig 2 and Table I and II) Thus an instillation of 170 mM lactic acid (L or D) did not in any respect influence the net gastric transmucosal ion transport on subsequent instillation of hydrochloric acid

TABLE III Electrolyte content in the fluid produced in unstimulated whole gastric pouches in cats in 30 min periods before and subsequent to a 30 min instillation of 6 ml of 700 mM L(+) lactic acid The values given are mean ± S E n = 3

Collection period	H^+ (μEq/30 min)	Na^+ (μEq/30 min)	K^+ (μEq/30 min)	Cl^- (μEq/30 min)	Lactate (μEq/30 min)	secretion rate (ml/30 min)
Control	20±10	93±2	5±1	115±11		0 7±0 1
post lactic acid						
0–30	127±17	321±87	37±20	377±51	111±6	2 4±0 6
30–60	26±9	226±51	9±1	214±33	11	1 6±0 3
60–90	36±59	280±78	15±6	353±151		1 9±0 5

(see Fig 5) The net influx of sodium ions increased and the net efflux of hydrogen ions decreased on increase in volume The net effluxes of hydrogen ions in the control period in HCl and in HCl_3 were corrected for the influence of changes in volume (Corrected H_{t} = Observed $H_{et} + k \Delta V$, k being the regression coefficient for the period in question calculated by the method of Bartlett (1949)) The thus obtained mean net effluxes ± S E at zero net change in volume for these periods were 482±43 511±33 and 608±12 μEq/30 min, respectively There was a statistically significant difference between the thus corrected net effluxes of hydrogen ions in HCl_3 and in the control period ($p < 0$ 05) When the mean regression coefficient for the net efflux of hydrogen ions for all instillations of HCl subsequent to an instillation of 700 mM L lactic acid (Fig 5) was used to calculate the net efflux of hydrogen ions at $\Delta V = 0$ the corrected net effluxes of hydrogen ions in HCl was calculated to be 541±33 and that in HCl_3 to be 577±14 μEq/30 min On comparison between these values and that in the control period (corrected as described above) no statistically significant difference was obtained (0 1 $< p < 0$ 5 and 0 05 $< p < 0$ 1 respectively) It must be considered that in both cases the corrections to zero net change in volume also involves an uncertainity due to extrapolation over a relatively large distance (Fig 5) and that the relation between H_{nt} and ΔV is not necessarily linear though linearity appears to be a good approximation when the changes in volume are relatively large

In 3 cats the basal electrolyte output in 30 min was determined and a 30 min instillation of 700 mM L(+) lactic acid was then given The stomach was then drained and the fluid produced collected at 30 min intervals The fluid produced was mucoid and as can be seen in Table III it contained mainly sodium and chloride ions (2 expts with D(−) lactic acid gave essentially the same result) As no rinsing with HCl was performed after the lactic acid instillation there was a large amount of lactic acid in the first collection period

L(+) lactic acid and gastr mucosal surface morphology

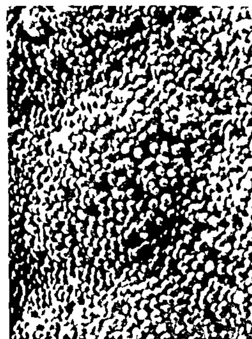
Specimens from 3 stomachs exposed to 6 ml of 700 mM L(+) lactic acid for 3 min and from untreated cor trol were examined in the scanning electron microscop

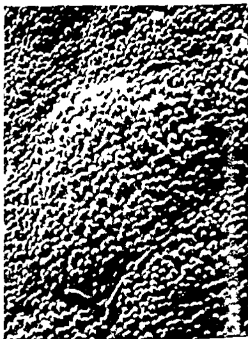




A B

Fig 6 A and B The appearance of gastric mucosal surface epithelial cells in the scanning electron microscope A is from an untreated control B from a mucosa fixed immediately after exposure to 700 mM L(+) lactic acid The cell surfaces are polygonal No separations in the intercellular junctions can be seen (Magnification ×2 250)





A B

Fig 7 A and B Surface epithelial cells at a higher magnification Numerous knob like microvilli are seen on the cell surfaces which are slightly convex upwards The cells appear closely attached to adjacent cells A control B post lactic acid (Magnification ×9 100)

In contrast to the findings in stomachs exposed to acetic or acetylsalicylic acid (Frenning and Öbrink 1971) no changes of the surface morphology of the gastric mucosa were found (Fig 6 A and B 7 A and B)

Discussion

According to our present theories and knowledge the prerequisite for a weak acid to change the permeability properties of the gastric mucosa is that it accumulates intracellularly to such a degree as to cause cellular swelling (due to an increased intracellular osmolality and/or to intracellular acidosis or to a specific action of its anion) and partial separations in the junctions between the mucosal cells and possibly also an increase in the intercellular spaces in the mucosa (Flemstrom and Frenning 1968 Flemstrom 1971 Frenning 1971 Frenning and Öbrink 1971, Flemstrom and Marsden 1972 *cf* Martin 1963) The results of Hingson and Ito (1971) who studied the fine structure of the surface epithelium in mouse stomachs exposed to some carboxylic acids also confirm the occurrence of cellular swelling subsequent to exposure to these acids They did not report any changes in the intercellular junctions until the process of cell degradation was far advanced Their results do not however exclude the possibility that incomplete separations in these junctions occurred as a consequence of cellular swelling as appeared to be the case in cat gastric mucosae exposed to acetic or acetylsalicylic acid on examination in the scanning electron microscope (Frenning and Öbrink 1971) Flemstrom (1971) observed an increase in the acid output from histamine stimulated frog gastric mucosa *in vitro* when it had previously been exposed to 10 mM L(+) lactic acid (pH = 4 00) added to the mucosal side solution No such effect was obtained subsequent to treatment with D(−) lactic acid or lactate (L or D pH = 7 12) His results strongly suggest qualitatively that L(+) lactic acid transiently accumulated in the mucosal cells and there is no reason to believe that the distribution of the D(−) isomer was different The gastric mucosal permeability for ions still remained unchanged after a 30 min instillation of 6 ml of 170 mM L(+) or D(−) lactic acid This would seem to indicate that the intracellular concentrations of lactate and hydrogen ions never reached such a magnitude as to cause cellular swelling The disappearance rate of 170 mM lactic acid from the stomach was lower than that of 170 mM HCl (see Fig 1 and 2) whereas 170 mM acetic acid disappears faster than HCl (Teorell 1939 Flemstrom Frenning and Öbrink 1964 Flemstrom and Frenning 1968) If lactic acid is less lipid soluble than acetic acid this may explain the results The partition ratio for L(+) lactic acid between isopropylether and 1 M HCl is considerably smaller than that for acetic acid (0 05 and 0 2 respectively Camien Fowler and Dunn 1959) which in fact indicates that lactic acid has a lower solubility than acetic acid in organic solvents For 3 barbituric acid derivatives with similar pK_a values Schanker *et al* (1957) showed that the higher the partition coefficient chloroform—HCl and heptane—HCl the higher was the disappearance rate from the stomach This may be due to different affinities of the mucosal lipids for the acids although it is possible that the affinity of the mucosa for weakly polar acids is not due to the lipids since the highly hydrophilic polysacharide dextran gels have high affinities for such solutes (Marsden 1972)

After exposure of the stomach to 700 mM L or D lactic acid it produced a mucoid fluid containing mainly sodium and chloride ions This caused a larger increase in sodium ion concentration and a larger decrease in hydrogen ion concentration on

subsequent instillations of hydrochloric acid in comparison with the control hydrochloric acid instillation It thus acted as a diluting secretion in the sense of the recent formulation of the two component hypothesis (Makhlouf, McManus and Card 1966) The net efflux of hydrogen ions was however, not increased on instillation of HCl subsequent to an instillation of 700 mM lactic acid (Table II) and further it decreased on increase in volume (Fig 5) These findings appear to exclude any neutralization of importance

Possible reasons for the reduction of the net efflux of hydrogen ions on increase in volume are that the fluid produced reduced the lumen to blood concentration difference and possibly also that the flow of fluid reduced the rate of diffusion of hydrochloric acid and into the gastric pits and tubules (*cf* Rehm Schlesinger and Dennis 1953) If the volumes of the recovered instillates were somewhat over estimated due to admixture of mucus the values for the net effluxes of hydrogen ions would have been falsely low and those for the net influxes of sodium ions falsely high Some contributory effects of this kind cannot be excluded The composition of the fluid produced after exposure of the gastric mucosa to 700 mM lactic acid (Table III) and the normal surface morphology of lactic acid treated gastric mucosa appear to exclude the possibility that acid secretion was stimulated and the composition of the secretion altered due to an increased diffusional transport over the mucosa (*cf* Frenning 1971)

On comparison of the present expts with 170 mM lactic acid and similar expts with 170 mM acetic acid (Flemstrom and Frenning 1968) it would appear that although both acids enter the cells (Flemstrom 1971) the amount of acetic acid that enters is sufficient to change the permeability of the mucosa for ions but the amount of lactic acid is not (for discussion of possible reasons to this vide supra) As there was no difference between the effects of the L and D isomers of lactic acid it is considered less probable that the difference in effect of acetic and lactic acids was due to a more rapid metabolism of lactate than of acetate

Not even on instillation of HCl subsequent to an instillation of 700 mM L lactic acid (*i e* in HCl) was the gastric mucosal permeability to ions increased In the following instillation period (HCl_3) the mucosal ion permeability was possibly increased—when correction was made to zero net flow of fluid the net efflux of hydrogen ions was possibly higher than that in the control period The explanation for this apart from the possibility that the value of H at ΔV = 0 was determined falsely high due to the uncertainty of this correction might be that though a relatively large amount of lactic acid probably entered the cells on instillation of 700 mM lactic acid no cellular swelling occurred because the luminal sides of the cells were exposed to a hyperosmotic solution On subsequent instillation of 170 mM HCl there might have been a moderate cellular swelling that increased the mucosal ion permeability in the next period

The present results further imply that an increased net influx of sodium ions into the gastric lumen in combination with an increase in volume (or a less than normal decerase in volume) taking place after any kind of instillation does not necessarily mean that the gastric mucosal permeability to ions is increased

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Acta physiol scand. 1972 85 374—389
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Excitability of the Acoustic m Stapedius and m Tensor Tympani Reflexes in the Nonanesthetized Rabbit

By

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Received 3 December 1971

Abstract

Borg E *Excitability of the acoustic m stapedius and m tensor tympani reflexes in the nonanesthetized rabbit* Acta physiol. scand 1972 85 374—389

Middle ear muscle responses to tone bursts (0 5 to 12 0 kHz) were studied in nonanesthetized unrestrained rabbits. The combined (total reflex) and the separate activity of the two muscles were recorded as changes of acoustic impedance in both ears simultaneously. The mean threshold of the ipsilateral total reflex, defined as the stimulus intensity eliciting 10 % of maximal response was 98 dB SPL at 0.5 kHz and fell 12 dB/octave up to 2 0 kHz. Above 4 0 kHz it was about 70 dB SPL. The m stapedius reflex was not found to differ from the total reflex, whereas the threshold of the m. tensor tympani reflex was about 10 dB higher at all frequencies. The crossed reflexes were significantly less sensitive than the ipsilateral reflexes. The stimulus-response curves of the m. tensor tympani were less steep than those of the m. stapedius and reached their maxima at a higher sound level. The time course of the responses was interpreted to show nonlinear properties of the reflex system. The sensitivity to acoustic stimulation in the rabbit was found to be significantly higher than in man. The relationship between the activities of the two muscles as well as differences between species are discussed

Descending and efferent pathways constitute an anatomically prominent part of the auditory system (for review see *e g* Filogamo *et al* 1967) through which various types of feedback are established. The middle ear muscles—m. stapedius and m. tensor tympani—are usually regarded as the most peripheral of the regulating mechanisms Together with the olivocochlear efferents they influence the response of the cochlea to sound stimulation.

In order to analyse the functional role of the middle ear muscles one should study quantitatively (a) the characteristics of stimuli activating the muscles (b) the influence of the muscle activity on the transmission of sound through the middle ear as a function of sound frequency and intensity (c) the dynamic properties of the muscle activity

The middle ear muscles have been known for long time to be activated in response to sound. They have recently also been shown to contract under a number of other conditions such as bodily movements (cat Carmel and Starr 1963 Simmons 1964 a) and vocalization (cat Simmons 1964 a bat Henson 1965 The properties of the

acoustic middle ear reflexes have usually been investigated on anesthetized decorticated or decerebrated animals (Lorente de No 1933, 1935 Lorente de No and Harris 1933 Wever and Vernon 1955 a 1955 b Eliasson and Gisselsson 1955 Wersäll 1958 Price 1963 1966) Under nonanesthetized conditions the middle ear reflexes have been studied with electrodes chronically implanted in the muscles of the middle ear or at the round window in cats (Galambos and Rupert 1959 Simmons 1959 1964 a Baust and Berlucchi 1964 Salomon 1966) and in acute experiments on immobilized rabbits (Kato 1913 Bornschein and Krejci 1952) In addition the acoustic reflexes as indicated by changes in the acoustic impedance of the ear have been investigated in the unrestrained unanesthetized rabbit (Borg and Møller 1968) and in man (see e g Møller 1962 b Jepsen 1963)

Experiments with electrodes chronically implanted in the muscles of the middle ear of cats have characteristically shown markedly different results and thus have not provided consistent quantitative information on the properties of the acoustic middle ear reflexes The discordance between results obtained in studies in the cat (Simmons 1959 1964 a 1964 c) and in man (Jepsen 1963) have not provided a basis for interpreting the function of the middle ear reflexes in the regulation of input to the cochlea (Simmons 1964 c) Studies using identical method to measure the middle ear reflex activity in animals and man would therefore be expected to contribute valuable information with regard to differences among species and the functional role of these reflexes Furthermore in past experiments on the control of sound transmission through the middle ear the two middle ear muscles have usually been regarded as a unit and therefore little information is available on their individual functions

The dynamic properties of the acoustic middle ear reflexes have been quantitatively analyzed in man (Møller 1962 a Dallos 1964) In animals restricted quantitative information is available but only from anesthetized or decorticate preparations (Lorente de No 1933 1935 Wersall 1958) Particularly oscillations in the reflex response to sound under certain circumstances have attracted attention and several attempts have been made to determine their significance (Lorente de No 1935 Eliasson and Gisselsson 1955 Wersall 1958) In man the oscillations have recently been shown to originate in the m stapedius feedback loop that serves to regulate the transmission of sound through the middle ear (Borg 1968 see also Møller 1962 a)

A series of experiments was aimed to quantitatively study the properties of the m stapedius reflex the m tensor tympani reflex and the combined or total reflex to sound The main object was to determine a) their excitability to pure tone stimulation b) their effect on sound transmission through the middle ear and c) the dynamic properties of their responses The middle ear reflex activity was recorded from both ears simultaneously as changes of acoustic impedance in nonanesthetized unrestrained rabbits by means of a recently developed method (Møller 1960 1961 Borg and Møller 1968 Borg 1972 a 1972 b)

The present article will present mainly quantitative results on the excitability of

the reflexes. It will also include a qualitative description of the time courses of the closed loop responses of the muscles, *i.e.* the response obtained under condition when the muscles influence the stimulus sound. A quantitative analysis of the dynamic properties is in press (Borg 1972 d).

Methods

Recording technique

The bilateral responses of the acoustic middle ear reflexes were recorded simultaneously as changes in the acoustic impedance at the eardrum by a method developed (Møller 1960, 1961) for human experiments. Application of the same technique in experiments on nonanesthetized rabbits has been described by Borg and Møller (1968). The technique as applied to rabbits has been evaluated with regard to intersession reproducibility and intrasession stability (Borg 1972 b).

The experiments were performed on 78 adult nonanesthetized rabbits as each remained quiet in a box open at the top. Rubber tubes were inserted in the ear canals and two identical impedance measuring devices were attached to these tubes. The impedance changes were measured simultaneously in both ears by a 800 Hz 65—70 dB SPL (re 0.0002 μb) explorer tone. The output of the measuring microphone was electrically balanced out when the muscles were at rest by adding an 800 Hz signal of equal amplitude and of opposite phase. The contraction of the muscles changes the impedance of the ear and upsets the balance resulting in an amplitude modulated 800 Hz signal. The rectified lowpass filtered (bandwidth 50 Hz, 18 dB/octave) impedance change signal is used as a measure of the middle ear muscle activity. Its steady state amplitude is proportional to the amplitude of the integrated EMG of the muscles (Borg 1972 a).

Reflex responses were elicted by 0.5 s bursts of pure tones with each burst having a rise and decay time of 2 ms (rise time to 90 % of steady amplitude and decay time to 10 % of steady amplitude). The tone frequencies used were 0.5, 1.45, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 kHz and the intensities ranged from below reflex threshold to about 125 dB SPL (re 0.0002 μb). Several stimulus frequencies were usually examined in each experimental session. In the beginning and at the end of such experimental sessions a series of 2.0 kHz stimuli were given to check the response stability (see Borg 1972 b).

The sound pressure level of the stimulus was measured in each ear with a probe that reached close to the eardrum. The sensitivity of the probe was calibrated with a Brül and Kjaer condensor microphone (Type 4134). The rubber tube used for the impedance measurements was coupled to the condensor microphone as a closed volume. The diaphragm of the condensor microphone had approximately the same position relative to the end of the probe as did the eardrum. The distance in the ear was less than 1 cm and in the probe calibration about 0.5 cm.

The response amplitudes at the end of the stimulus were measured and expressed in percentage of maximal obtainable amplitude. The curves showing responses in the right ear were calculated as percentages of the maximum response obtained in the right ear to ipsilateral stimulation. The curves for the left ear were calculated as percentages of the maximum response obtained in the left ear. Four stimulus-response curves were calculated for each stimulus frequency, two for ipsilateral and two for contralateral stimulation. At frequencies where a definite maximum was not obtained the maximum value for 2.0 kHz was used. This procedure was applied for frequencies above 4.0 kHz and sometimes at 0.5 kHz. At frequencies above 4.0 kHz the sound intensity was seldom high enough for the responses to reach a maximum due to the limitations of the stimulus earphone. At 0.5 kHz the stimulus artifact at the highest intensities sometimes made determinations of maximal amplitudes uncertain.

Surgical procedures

In order to record the isolated m. tensor tympani activity the m. stapedius was inactivated by denervation. The denervation was performed in eight rabbits by extracting the 7th cranial nerve through the facial canal after carefully dissecting the nerve free from the stylomastoid foramen. In 5 animals the m. stapedius was denervated bilaterally, in two cases this was done in one session. The nerve was interrupted central to the stapedial nerve branch thus causing a complete paralysis of the m. stapedius (Kato 1913). Even though convincing evidence is lacking regarding sensory innervation to the m. stapedius (see further Blevins 1964, Wigand and Brauer 1964), the denervation was made to interrupt any such connections. In 10 other rabbits the facial nerve was transected in the brain stem at the level of the internal geniculum.

In these cases the crossed olivo-cochlear efferents were also cut Since the crossed efferents influence the afferent auditory activity in the medium and high frequency range of the rabbit (Borg 1971) these animals were used only in that portion of the study dealing with properties at 0 5 kHz.

The isolated m stapedius reflex was studied after cutting the m. tensor tympani The bulla was reached from the ventral side and opened with a knife and a curved tipped hemostate Although several attempts were made to cut the 5th cranial nerve containing the motor fibers to the m tensor tympani this procedure failed due to profuce bleeding from veins anterio-lateral to the bulla The threshold values of reflexes elicited from the ears with the deactivated m tensor tympani were in some cases increased 10—20 dB one to two days postoperatively This deterioration was probably not due to damage to the hair cells since the cochlea appeared normal in surface preparations made from two such cases[1] In some of the cases in which the reflex thresholds were raised the middle ear was reopened two or three days after cutting m. tensor tympani A clear yellow exudate was found partly filling the middle ear On later occasions small masses of mucous were found attached to the ossicles This exudate was probably related to inactivity of the m tensor tympani since the reflex properties in two control animals were observed to be unchanged when measured several times after operations in which the m tensor tympani was manipulated without being cut Only those animals were included in which the hearing was unchanged on the operated side as measured by the crossed middle ear reflex response (14 animals)

Results

Dynamic properties

The middle ear reflexes constitute a feedback system controlling the sound transmission through the middle ear Under closed loop conditions the contraction of the muscles influences the transmission of the stimulus sound Under open loop conditions transmission of the stimulus sound is not influenced *e g* when the muscles in the stimulated ear are inactivated The response of a closed loop feedback system is often characterized by damped oscillations Measurement of the frequency relative amplitude and degree of damping of the oscillations provides information relevant to the dynamic properties and the stability of the system In this case it is important to determine if the system is linear if linear the system can be analyzed and evaluated on the basis a relatively small number of such measurements In a linear system the shape of responses to step stimuli of various intensities is similar differing only in amplitude Furthermore the time courses of the responses are the same to a positive as well as a negative step stimulus (*e g* the onset and the decay of a tone burst)

The total reflex and the isolated m stapedius reflex

Fig 1 shows examples of the total reflex *i e* middle ear reflex responses with both middle ear muscles intact (left column) and responses with only the m stapedius intact (right column) to ipsilateral stimulation with pure tone of 2 0 kHz at various intensities (legend numbers) In other words the recordings in Fig 1 show closed loop responses from the same ear before (left) and after (right) sectioning the m. tensor tympani The maximal response in both columns has been normalized to equal amplitude The beginning and the end of the stimulus tone burst appears often in the ipsilateral recordings (Fig 1 and Fig 2) as a pulse of varying amplitude

[1] Surface preparations made by Miss B Engstrom Dept Oto rhino laryngology Akademiska Sjukhuset Uppsala

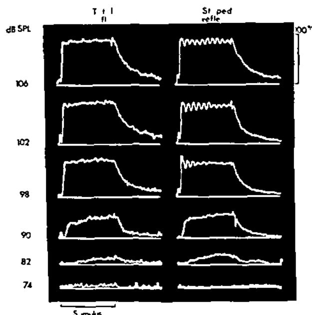


Fig 1 Middle ear reflex responses recorded as changes in the acoustic impedance of the ear Ipsilateral stimulation with 2 0 kHz pure tone of various intensities in a nonanesthetized rabbit with both middle ear muscles intact (total reflex left column) and with only m stapedius intact one month after cutting the m tensor tympani (right column) Maximal response is normalized to 100 per cent in each case Stimulus duration was 0 5 s (scale lower left)

It is to be noted that the relative amplitudes of the responses are not changed significantly by sectioning of the m tensor tympani

The oscillations however are much more prominent in the isolated m stapedius response than in the response with both middle ear muscles intact (total reflex) It should be pointed out that the oscillations in the total reflex response are generally more pronounced than shown in Fig 1 (left column 102 dB SPL) Thus the difference in terms of oscillation between the total reflex and the isolated m stapedius reflex is generally less than shown in Fig 1

The oscillations are more or less pronounced in the responses of the m stapedius reflex and of the total reflex in nearly all the rabbbits investigated The oscillations were most prominent with low frequency ipsilateral stimulation (0 5 to 2 0 kHz) in the intensity range from 100 to 115 dB SPL Below the relative response amplitude of 70—80 % (at 2 0 kHz) the oscillations were absent and they were found to decrease at maximal stimulation (above 115 dB SPL at 2 0 kHz)

The oscillations usually were of a damped character and the maximal peak to-peak amplitude seldom exceeded 20 per cent of the maximal impedance change and 30 per cent of the individual response amplitude. Limit cycling (*i e* continuous oscillations of constant amplitude) was observed in the isolated m stapedius response as it has been sometimes observed after moderate doses of anesthetics The frequency of the oscillations was usually 18—22 Hz at 2 0 kHz. The degree of damping decreased and the frequency of oscillation usually increased as sound intensity was raised

For 0.5 kHz stimulation oscillations were frequently observed in responses with amplitudes down to 40 per cent of maximum In such cases the frequency of oscillation was lower than for 2 0 kHz stimulation even below 10 Hz.

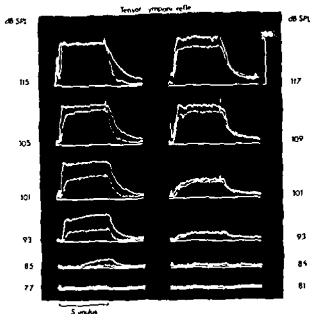


Fig 2 Responses recorded as changes in acoustic impedance of *the ear Stimulation with 0 5 s* duration bursts of 2 0 kHz pure tone (scale lower left)

Left column Ipsilateral responses of the total reflex (continuous line) and the m tensor tympani reflex (one week after denervation of the m stapedius broken *line) in the same ear of a non* anesthetized rabbit Maximum response amplitude is normalized to 100 per cent and the recordings *are photographically super* imposed

Right column Simultaneous responses of the ipsilateral (continuous line) and contralateral (broken line) m tensor tympani reflex in another rabbit

The oscillations were usually less prominent in the contralateral response yet the frequency was always the same as in the ipsilateral response The oscillations in the initial part of the crossed reflex response were often somewhat depressed probably due to a slightly longer rise time making the damped character less clear On certain occasions they were observed to increase in amplitude during the initial part of the response

The system supplying the responses shown in Fig 1 cannot be considered as linear Several signs of nonlinearity are indicated Firstly the rise times of the responses are faster than the decay times except at lowest intensities Secondly the time course and the rise time appeared to be dependent on the intensity level of the stimulus The rise time decreases and the oscillations appear when the sound intensity is raised above a certain value On the other hand the decay times show a tendency to increase as a function of the intensity level of the stimulus

The m tensor tympani reflex

Denervation of the m stapedius was followed by prominent changes in the temporal pattern of the impedance changes elicited by pure tone stimulation Fig 2 shows in the left column a typical example of the ipsilateral impedance change before (continuous line) and one week after (broken line) denervation of the m stapedius (the isolated ipsilateral m tenor tympani reflex) It is to be noted that the m tensor tympani reflex (broken line) has a longer latency and a longer ri e time but a faster decay after the end of the stimulus than the total reflex (continuous line) to the same stimulus intensity The time course of the rise and the decay of the m tensor tympani response is nearly identical Oscillations (frequency 8—12 Hz) were rarely

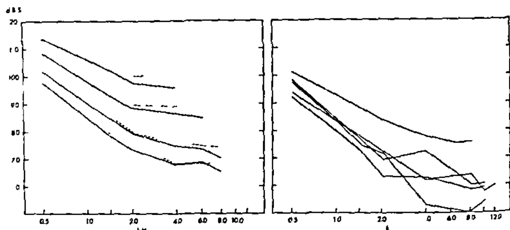


Fig 3 *Left graph* Sound intensity giving 10 20 50 80 % of maximal impedance change in the ipsilateral ear (continuous line) and the contralateral ear (broken line) based on recordings in 78 rabbits with both middle ear muscles intact (total reflex)

Right graph Ipsilateral reflex "threshold curves (sound intensity giving 10 % of maximal impedance change) of total reflex for 5 individual nonanesthetized rabbits

Bursts of pure tones 0 5 s in duration were used as stimuli

observed in the isolated m tensor tympani response The right column of Fig 2 shows the m tensor tympani response to ipsilateral (continuous line) and contralateral (broken line) stimulation in a rabbit with bilateral m stapedius paralysis The shape of the responses are seen to be similar to both ipsilateral and contralateral stimulation The onset of the crossed reflex is slightly slower than the ipsilateral response which is in accordance with the observations for the total reflex The two methods used for denervation of the m stapedius gave very similar results with respect to the time course of the m tensor tympani response

Excitability

The total reflex

Fig 3 (left graph) shows the sound pressure levels necessary to obtain reflex responses of 10 20 50 and 80 % of the maximal amplitude for stimulation with frequencies from 0 5 to 8 0 kHz The average of the ipsilateral (continuous line) and the contralateral (broken line) reflex responses from 78 rabbits are shown The reflex threshold (10 % of maximal response) decreased at a rate of about 12 dB per octave up to 2 0 kHz Between 4 0 and 8 0 kHz it was nearly constant at 70 dB SPL The excitability of the ipsilateral response was usually slightly higher but the difference especially at the high stimulus frequencies was small At 0 5 and 2 0 kHz the average difference was 1 7 and 1 8 dB respectively Both were highly significant ($p \leq 0\,0001$) The dynamic range (10 % to 80 % of maximal response) was about 15 dB for the low frequencies (0 5 kHz) where the reflex threshold was high the dynamic range was 25 to 30 dB for the high frequencies (above 2 0 kHz) Thus the

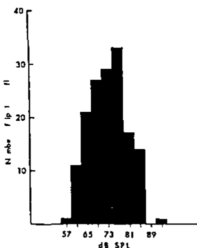


Fig 4

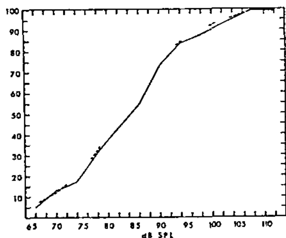


Fig 5

Fig 4 Frequency histogram showing sound intensity giving 10 % of maximal impedance change in 154 ipsilateral reflexes from 78 rabbits Stimulation with 2 0 kHz pure tone of 0 5 s duration

Fig 5 Stimulus response relation of the ipsilateral total reflex (broken line) and the m stapedius reflex recorded in the same ear three weeks after cutting the m tensor tympani (continuous line) Stimulation with 2 0 kHz pure tone of 0 5 s duration.

maximal response amplitudes at the various frequencies were attained within a narrower intensity range than were the threshold responses As a rule the ipsilateral stimulus response curves reached a plateau for stimuli below 115 dB SPL (2 0 kHz) The maximum response amplitude for ipsilateral stimulation was at the most a few per cent above that for contralateral stimulation

Fig 3 (right graph) shows the sound level necessary for 10 % impedance changes at various frequencies for 5 rabbits The general shape of the threshold curves is similar the level of excitability however varies between animals The lowest threshold level ever observed was 44 dB SPL (at 4 0 kHz)

The individual variability of the sound level that produced 10 % of maximal impedance change to ipsilateral stimulation at 2 0 kHz is shown in Fig 4 Each ear is represented by the best available estimate of its average threshold based on several determinations in each case The range was nearly 40 dB the mean value was 73 1 dB SPL and the standard deviation was 7 0 dB The reproducibility within the same animal was however good (Borg 1972 b)

The isolated m stapedius reflex

Fig 5 shows a typical example of the stimulus response curve of the ipsilateral middle ear reflex before (broken line) and three weeks after tendotomy of the m. tensor tympani (continuous line) It is seen that the stimulus response curve was not influenced by cutting the m tensor tympani The small difference between the two curves in Fig 5 is well within the range of normal variation Since evidently the influence of the m tensor tympani on the total relative impedance chang

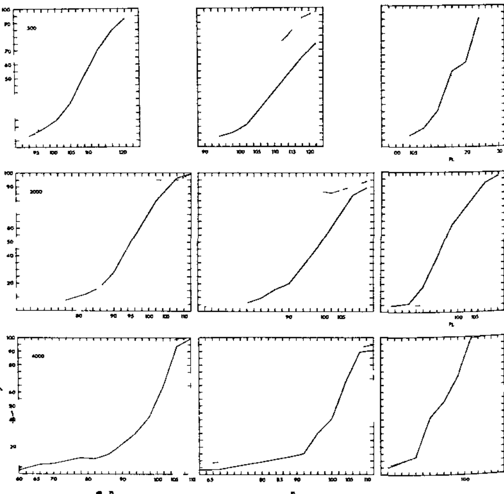


Fig. 6. Stimulus response curves of the middle ear reflex responses obtained as changes in the acoustic impedance of the ear. Stimulation with pure tones of 0.5 s duration at 0.5 kHz (upper row), 2.0 kHz (middle row) and 4.0 kHz (bottom row). The responses are normalized to 100 % using the maximal ipsilateral response obtained at 2.0 kHz as the reference.

Left and middle columns show the ipsilateral and the contralateral stimulus response curves respectively before (broken line) and after (continuous line) bilateral denervation of the m. stapedius. Recordings in one ear of a nonanesthetized rabbit with two months interval.

Right column shows the ipsilateral responses of the isolated m. tensor tympani (continuous line) and the responses in the same ear to stimulation in the other ear where the m. stapedius and m. tensor tympani are intact (broken line).

insignificant the excitability properties shown in Fig. 3 for the total reflex represent in large measure the excitability of the m. stapedius reflex. Below the threshold of the m. tensor tympani reflex (see next section) for values referring to 10 % and 20 % impedance change the agreement is precise.

The isolated m. tensor tympani reflex

The stimulus response relationship obtained after denervation of the m. stapedius represents the excitability properties of the isolated m. tensor tympani reflex. It is

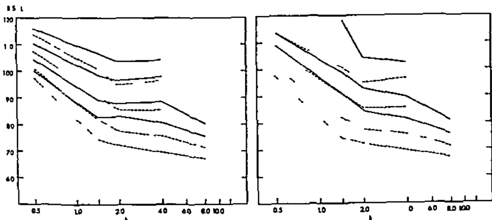


Fig 7 Sound intensity giving 10 20 50 and 80 % of maximal impedance change at various stimulus pure tones of 0 5 s duration Averages of recordings in 8 rabbits

Left graph Ipsilateral m tensor tympani responses (continuous lines) and the responses from the same ears before denervation of the m stapedius (broken lines)

Right graph Crossed isolated m tensor tympani responses with stimulation of the ear having both muscles intact (continuous lines) and the ipsilateral responses before denervation (broken lines)

however more important to know the characteristics of the ipsilateral m tensor tympani reflex when activated together with the m stapedius In order to know that the relation between the ipsilateral and the contralateral isolated m tensor tympani reflex must be determined Then the properties of the ipsilateral m tensor tympani reflex *in conjunction with that of the m stapedius can be calculated from* studies of the crossed isolated m tensor tympani response elicited from the ear with both mucles intact

The left and middle columns of Fig 6 show typical examples of stimulus response curves of the ipsilateral and contralateral reflex respectively before (broken line) and after (continuous line) bilateral denervation of the m stapedius in one rabbit It is seen that the excitability of the m tensor tympani was lower than that of the total reflex The difference was especially prominent at higher frequencies There was no major change in the shape of the curves even though the stimulus response curves sometimes had steeper slope after denervation (see the curves at 4 0 kHz) A comparison of the solid lines of the left and the middle columns shows that the shape of the ipsilateral and contralateral m tensor tympani stimulus response curves was about equal (see also Fig 2) On the average 10 % of maximal response was obtained at a 1 1 dB lower intensity (standard deviation = 3 5 dB) for the ipsilateral than for the contralateral m tensor tympani reflex in four rabbits with bilateral m stapedius paralysis *The corresponding difference at 50 % was 3 2 dB* (standard deviation = 3 2 dB)

The right column of Fig 6 shows examples of stimulus response curves from a rabbit with unilateral m stapedius paralysis The stimulus response curves of the m tensor tympani reflex in the ear with m stapedius paralysis were obtained by ipsi-

lateral (continuous line) and by contralateral (broken line) stimulation i e stimulation of the ear with both muscles intact The m tensor tympani reflex had a higher threshold and the response amplitude was smaller when the m stapedius was contracting simultaneously than when m tensor tympani was activated alone The difference in response was most prominent at low frequencies Since there was only a slight difference in excitability between the ipsilateral and contralateral responses of the isolated m tensor tympani reflex (see above) the crossed response of the isolated m tensor tympani reflex elicited by stimulation in the ear with both muscles intact (Fig 6 right column broken line) closely represents the activity of the m tensor tympani in the stimulated ear as well

Fig 7 shows a summary of measurements on the excitability of the isolated m tensor tympani reflex in eight rabbits The continuous lines in Fig 7 show in the left graph the ipsilateral m tensor tympani reflex when the m stapedius is denervated and in the right graph the crossed m tensor tympani reflex with the m stapedius intact on the stimulus side The broken lines in Fig 7 left and right represent the excitability of the ipsilateral total reflex in the same rabbits before denervation of the m stapedius

The ensitivity of the isolated ipsilateral m tensor tympani reflex was about 10 dB lower than that of the total reflex in the range above 2 0 kHz while the difference at 0 5 kHz was only 3 dB There was a tendency toward a steeper slope of the stimulus response curves for the m tensor tympani The excitability of the m. tensor ympani reflex at 0 5 kHz after cutting the facial nerve in the floor of the fourth ventricle did not differ from the values shown in Fig 7

As pointed out in connection with Fig 6 the crossed isolated m tensor tympani reflex elicited from the ear with both muscles intact disclosed the properties of the m tensor tympani reflex when acting in conjunction with the m stapedius Fig 7 (right) shows that the threshold of the m tensor tympani reflex was about 10 dB less than that of the m stapedius reflex at all frequencies tested Furthermore the rate of increase in impedance change as a function of the level of intensity for the m tensor tympani was much slower than for the total reflex (m stapedius reflex) At 1 45 kHz the difference between 10 % and 50 % response amplitude was 27 dB and 16 dB for the m tensor tympani and the m stapedius respectively

Discussion

The excitability of the m stapedius and m tensor tympani reflexes

The present experiments indicate that the threshold of the m stapedius reflex in unanesthetized rabbits was about 10 dB lower than that of the m tensor tympani reflex up to 8 0 kHz This value refers to stimulation with pure tones Noise stimulation has been found to be a more effective stimulus than pure tones in man (Møller 1962 a Flottorp *et al* 1971) and to lower the reflex threshold by up to about 20 dB There is however no reason to postulate a differential effect on the muscles in this respect The threshold of the isolated m tensor tympani reflex (m stapedius

paralyzed) was at low frequencies (0 5 kHz) only slightly higher (3 dB) than that of the total reflex of the same ear (*i e* m stapedius Fig 7 left graph) Due to the large attenuation of sound transmission provided by the m stapedius at this frequency (Borg 1972 c) the difference in excitability is maintained independent of the sound frequency throughout a wide frequency range

The low excitability for the m tensor tympani reflex is in agreement with earlier studies in anesthetized or restrained rabbits (Kato 1913 Lorente de No and Harris 1933 Wersall 1958) Lorente de No and Harris (1933) found however usually identical thresholds above 2 0 kHz In the anesthetized cat the excitability as judged from the electromyographic activity is 30 dB lower for the m tensor tympani than for the m stapedius (Eliasson and Gisselsson 1955 see however Wever and Vernon 1955 a) As for the nonanesthetized cat quantitative data is not available but it can be inferred from Carmel and Starr (1963) that the m stapedius has a slightly lower threshold than the m tensor tympani on the order of a few dB

The stimulus response curve of the m tensor tympani reflex when the latter is active simultaneously with the m stapedius reflex was less steep and had the maximal response amplitude at a higher intensity than the m stapedius The same relation between the maxima of the reflexes was found by Wersäll (1958) who measured muscle tension in acute experiments The functional organisation of the middle ear reflex system in the rabbit thus implies that the m tensor tympani reflex increases the dynamic range of the total middle ear reflex activity However the importance of this effect seems to be small below 120 dB SPL in the frequency range below 2 0 kHz (Borg 1972 c)

Species differences in the excitability of middle ear reflexes

A certain physiological method is for technical reasons often applicable only to a limited number of species It can therefore be difficult to judge if an observed difference is due to variations in technique or differences among species

The results of the present study in the rabbit were obtained with a method that has also been used in man under closely identical circumstances (Moller 1962 b Borg unpublished) It is thus possible to determine if differences exist in these two species *e g* with respect to sensitivity of the middle ear muscles to acoustic stimulation The ipsilateral m stapedius reflex threshold (10 % of maximal impedance change) has been determined in 17 separate experiments on 10 human subjects at 2 0 kHz The threshold showed values between 86 and 102 dB SPL (Borg unpublished) Moller (1962 b) found threshold values between 88 and 98 dB SPL in four subjects The average reflex threshold observed at 2 0 kHz in the rabbit (73 dB SPL) thus differs significantly ($p \leq 0\ 001$) from even the lowest threshold values observed in human experiments In the frequency range above 2 0 kHz the sensitivity of the middle ear reflexes is 15—20 dB higher in rabbit than in man (see Moller 1962 b) At 0 5 kHz however the difference is small (see Moller 1962 b) The small difference at 0 5 kHz may be related to the fact that the threshold of hearing is relatively higher in this range in the rabbit than in man (Price 1

Thus there appears to be a clearcut difference between man and rabbit with respect to the excitability of the middle ear reflexes

Results obtained in the cat are much more difficult to judge Some studies in non anesthetized cats have resulted in excitability values similar to those in rabbits (Carmel and Starr 1963, Marsh and Worden 1969) Simmons however has observed remarkably low threshold values at 1 0 kHz varying from about 20 dB (re human audibility threshold 1959) to 50—60 dB SPL (four of the more sensitive preparations 1964 b) Most recordings in nonanesthetized cats have been performed with chronically implanted steel electrodes in the middle ear In this sense it is important to note that the effects of the presence of electrodes implanted in the middle ear have not been thoroughly investigated For example chronically implanted electrodes in the m stapedius tendon of man have been found to give rise to a sensation of noise (Salomon and Starr 1963) It should al o be noted that the electrical activity of the retina is dramatically changed by steel particles implanted in the vitreous body of the eye (Knave 1970) The magnitude and the direction of change were found to be dependent on the composition of the alloys

Thus at least for the moment there is little reason to postulate that the average excitability values differ significantly between the cat and the rabbit On the other hand the difference in the stability of the responses as obtained in these two species are more apparent In the cat the middle ear reflex responses appear to adapt rapidly to continuous stimuli at moderate intensity levels even at low frequencies (Simmons 1963) In the rabbit the responses appear to remain constant during prolonged stimulation (Borg and Møller 1968) Lorente de No (1935) was the first to point out this difference between the cat and the rabbit Wersall (1958) found that during chloralose anesthesia the middle ear muscles of the cat respond with a single twitch whereas they respond with sustained oscillatory activity in the rabbit

Habituation to repeated stimuli was investigated in the cat by Simmons and Beatty (1964) they showed that the reflex threshold could increase by as much as 50 dB during two hour long experimental sessions According to Baust and Berlucchi (1964) the habituation of the m tensor response of the cat is complete within 5 to 20 minutes This variability is in contrast to the stable results obtained in non anesthetized rabbits where the excitability in control measurements at the end of experimental sessions of about one hour showed an average decrease of only 1 2 dB (Borg 1972 b) from the original value It should be noted that such rapid changes in the responses to sound as shown in the cat with chronically implanted electrodes are very similar to the habituation of the startle response to sound (Hoffman and Searle 1968)

The importance of startle reactions as a basis for responses of middle ear muscles has been emphasized in studies of the m tensor tympani activity in man In man the m tensor tympani does not regularly respond to sound A generally held opinion largely based on work by Klockhoff 1961) maintains that the m tensor tympani can only be activated as a part of a startle response Klockhoff (1961) showed that jets of air directed towards the ocular region elicited an impedance change in normal

ears and in ears devoid of normal m stapedius function It was suggested that both muscles take part in the response a point later verified with electromyographic recordings (Djupesland 1967) The effective stimuli also caused bilateral contractions of the face and neck muscles and thus were concluded to be of startle nature A major characteristic of the startle response of the middle ear muscles in man was its rapid habituation It was later shown by Djupesland (1967) that auditory stimuli could also activate the m tensor tympani provided they produced a startle reaction It thus seems important to differentiate between specific acoustic reflexes and more generalized arousal and startle reactions

Furthermore in the cat the excitability of the middle ear reflexes differs considerably between successive measurements with intervals varying from days to months (Simmons 1963) Only considerable effort (*e g* anesthetizing the animals after each experimental session Simmons 1964 b) can keep the from time to-time variability below ±5 dB (*cf* Simmons 1963) In the rabbit the reproducibility including the first experimental session is within a few dB as determined from repeated routine experiments (Borg and Møller 1968 Borg 1972 b) It is good also in man (Møller 1962 b *Feldman and Zwislocki 1965*)

Thus it can be concluded that differences among species exist with regard to both absolute level of excitability and variance of responses of the middle ear reflexes This species variability probably serves to indicate fundamental differences in the function of the auditory system related to the behavior of the animals and man and their respective normal acoustic environment Thus it should be pointed out that great care has to be taken in using the results from animal experiments as the basis for hypotheses about the function of the human middle ear reflex

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From the Department of Physiology Karolinska Institutet Stockholm Sweden

Effects of Sympathetic Stimulation on Mechanoreceptors of Cat Vibrissae

By

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Abstract

Nilsson B Y *Effects of sympathetic stimulation on mechanoreceptors of cat vibrissae* Acta physiol scand 1972 *85* 390—397

In a previous investigation adrenergic nerve terminals were demonstrated in the hair follicles of cat vibrissae The present study shows that electrical stimulation of the cervical sympathetic trunk produces small but repeatable changes in the dynamic responses of the slowly adapting mechanoreceptors of the vibrissae in that the number of impulses in each discharge is reduced and their latency increased Changes in the static responses are very small These effects were abolished by phentolamine The receptor responses as well as effects of sympathetic stimulation remain unaffected by a brief carotid occlusion It is concluded that the sympathetic modulation observed is a direct effect of locally released noradrenaline and is not attributable to circulatory changes or to pilomotor activity

That activity in sympathetic nerves might influence the input from sensory receptors was suggested as early as in 1851 by Claude Bernard but only few experimental results showing a direct sympathetic effect at the receptor level have been reported Loewenstein (1956) showed that electrical stimulation of the sympathetic nerve supply to frog skin alters the responses from tactile receptors lowering their threshold and slowing adaptation (*cf* also Chernetski 1964) In mammalian muscle spindles stimulation of sympathetic fibers causes a slight facilitation followed by an increase in threshold (Hunt 1960) However results in favor of a sympathetic control of mammalian skin receptors do not seem to have been published previously

In studies of skin pieces from different kinds of mammals using Falck and Hillarp s fluorescence method Falck and Rorsman (1963) found no adrenergic nerve terminals in hair follicle In cat skin however Fuxe and Nilsson (1965) found the so called sinus hairs to be an exception They could demonstrate a rich adrenergic innervation of the hair follicles of the vibrissae in the face as well as of the carpal tactile hairs of the foreleg In previous studies of the carpal sinus hairs (Nilsson and Skoglund 1965 Nilsson 1968 1969) stimulation of the sympathetic nerve supply to

this skin area was shown to induce a vigorous contraction of the pilomotor musculature around the sinus hair follicles Since however the piloerection in itself changes the responsiveness to mechanical stimuli it is impracticable to study a possible direct effect of sympathetic activity on the mechanoreceptors of these sinus hairs Hence interest has now instead been focused on the vibrissae which have a striated musculature in the immediate vicinity of the hair follicles (Vincent 1913)

The aim of the present study has been to examine how the slowly adapting hair follicle receptors of cat vibrissae may be influenced by electrical stimulation of the cervical sympathetic trunk and by intravenous injections of noradrenaline

Methods

5 cats weighing 1 8—4 0 kg were used The animal was anesthetized with Nembutal 40 mg/kg b wt i.p and the anesthesia could be maintained by injecting small amounts of the same drug through a catheter in the femoral vein The cat was kept warm with an infrared heating lamp above the experimental table

After introduction of a tracheal cannula the left vagosympathetic trunk was dissected free The nerve trunk was cut caudally and placed on silver wire bipolar stimulating electrodes Square wave pulses of 1 ms duration were used and the stimulus strength was adjusted so as to give a strong retraction of the nictitating membrane and a dilatation of the pupil at a stimulus rate of 5—10/s

The cat s head was rigidly fixed in a metal head holder After enucleation of the left eye the infraorbital nerve was exposed by dissection in the floor of the orbital cavity and covered with liquid paraffin *Fine nerve filaments were dissected free and placed on a chlorided silver* wire electrode another electrode of the same type being in contact with inactive tissue The action potentials were amplified displayed on one beam of the oscilloscope and photographed After isolating a slowly adapting sensory unit that could be activated by deflecting one of the vibrissae on the upper lip manual probing established the direction in which the unit was most sensitive to hair deflection Precise displacements in this direction could then be achieved by means of a mechanical stimulator consisting of a rod glued to the center of a loudspeaker cone (for details see Nilsson 1969) The movement of this stimulating rod was usually perpendicular to the hair and parallel to the skin surface the distance from the skin being 3—5 mm The hair was deflected at a constant rate to a new position at which it was kept for a preset period of up to one s Stimulus velocities from 8 mm/s up to 80 mm/s were used The movement of the rod was recorded by a capacitance meter and displayed on the second beam of the oscilloscope By observing the hair movement through a microscope it could be checked that the time intervals between successive mechanical stimuli were long enough to permit the passive return of the hair to its initial position

Results

In the absence of induced movements of the hairs the vibrissae units were usually silent in a few cases a spontaneous activity could however be recorded Deflection of the hair with the mechanical stimulator produced a series of nerve impulses the frequency of which depended on the movement velocity During the following static plateau phase when a constant deflection was maintained a slowly adapting impulse discharge was recorded the frequency of this discharge was related to the displacement amplitude No detailed analysis of the sensitivity of the vibrissae receptors to different types of mechanical stimuli was made in this study but the results do not differ essentially from those obtained in earlier investigations on the vibrissae of cat (Fitzgerald 1940 Hahn 1971) and rat (Zucker and Welker 1969)

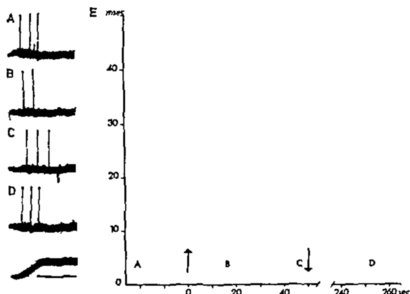


Fig 1 *A D* Sensory unit responses to rapid deflections of cat vibrissa before *(A)* and during stimulation of cervical sympathetic trunk at 10/s *(B* and *C)* *D* about three min after end of stimulation initial response is recovered Below recording of movement Time bar 50 ms *E* graphical representation of latencies from onset of each mechanical stimulus to first (○) second (●) and third (□) dynamic spikes Mechanical stimuli (velocity 14 4 mm/s amplitude plateau 450 μm) delivered at 2 5 s intervals Arrows show onset and end of sympathetic stimulation Times for recordings in *A D* indicated

The effect of sympathetic stimulation on the dynamic responses Precisely defined brief mechanical stimuli were applied at time intervals of 2 5 s As a rule the movement velocity and the plateau amplitude were chosen so that two or three impulses were recorded in the dynamic phase After a control period establishing that the responses displayed only insignificant variations electrical stimulation was applied to the cervical sympathetic trunk Recordings from a typical experiment are shown in Fig 1 During the control period *(A)* the movement results in 3 spikes with latencies of 8 22 and 32 ms respectively from the initiation of the movement When stimulation has been applied for 15 s *(B)* the response changes in ofar as the *same movement now produces only 2 spikes The latency of the first discharge* has increased to 12 ms but the time interval to the second spike is essentially unchanged In this experiment the sympathetic stimulation was maintained for 50 s the third spike reappeared occasionally *(C* at 45 s) but the interval between the second and third spikes was then longer than before About three min after cessation of the sympathetic stimulation *(D)* the response to the mechanical stimulus is once more the same as in the control period

In this experiment 10 to 15 s elapsed before any effect of the sympathetic stimulation could be observed this appears from Fig 1 *E* which is a graphical representation of the latencies of the three spikes calculated from the beginning of the displace

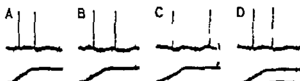


Fig 2 Deflection of vibrissa at 14 4 mm/s up to an amplitude plateau of 255 μm gives two dynamic spikes (A) During sympathetic stimulation at 10/s spike interval increases (B at 17 5 s C at 27 5 s) D three min after end of stimulation initial response is recovered Time bar 20 ms

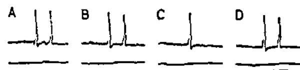


Fig 3 Pushing vibrissa into the skin (velocity 14 4 mm/s amplitude plateau 50 μm) gives two dynamic spikes (A) Response in B 12 5 s and in C 22 5 s after onset of sympathetic stimulation at 10/s D three min after end of stimulation initial response is recovered Time bar 5 ms

ment During the next 15 s there is a gradual increase in spike latencies but then a comparatively steady state seems to have been attained The delay from onset of sympathetic stimulation to the first change in response varied between 10 and 25 or occasionally 30 s in different experiments In one experiment this delay increased from 20 to 30 s when the stimulus rate was lowered from 10 to 5/s The response had usually recovered its initial pattern about 1 min after the end of the sympathetic stimulation

In similar studies of several vibrissae units the effect of the sympathetic stimulation was found to vary somewhat In some experiments responses of the type shown in Fig 1 were obtained i.e. spike latencies increased and the number of spikes in each discharge was reduced In other cases the spike intervals increased without any impulse being blocked as in Fig 2 In still other experiments the last spike in the dynamic phase disappeared without any preceding apparent change in latency and in some cases no effect whatever could be observed

The effects were the same regardless of whether the hair deflection was parallel or perpendicular to the upper lip and also when the movement was perpendicular to the skin surface i.e. when the stimulator pushed the vibrissa into the skin Fig 3 illustrates an experiment of this latter type in which the second discharge was blocked and the latency of the first impulse slightly increased

By changing the rate of the sympathetic stimulation it could be established that frequencies between 5 and 10/s had a clear and repeatable effect whereas stimulus rates below 2—3/s never resulted in any perceptible changes in the dynamic response pattern Nor had intravenous injections of noradrenaline in amounts of 1 5—5 0 μg/kg b wt any effect on these responses

The effect of sympathetic stimulation on the static responses It was more difficult to demonstrate a sympathetic influence on the impulse responses during the first

second of the static plateau phase When the mechanical stimulus was of low amplitude resulting in only a few impulses during this phase these discharges were ometimes blocked by the sympathetic stimulation On the other hand when a stronger mechanical stimulus induced a high frequency plateau discharge no changes whatever could be observed in the response pattern Following intravenous injections of noradrenaline (1 5—5 0 μg/kg b wt) the number of spikes decreased in two cases while 5 other units were unaffected

In some experiments the vibrissa was kept deflected (or slightly pushed inward) by the mechanical stimulator for somewhat longer periods After a few minutes the response had then adapted to an irregular low frequency discharge of 1 up to 5 imp/s On sympathetic stimulation a further decrease in frequency or even a *blocking of the discharge resulted These effects set in earlier if the stimulation was* repeated after half a minute s break In one case intravenous injection of nor adrenaline (3 μg/kg b wt) slightly increased the adapted impulse discharge

General observations The effects of sympathetic stimulation on the dynamic and static responses described above disappeared entirely following an intravenous in jection of phentolamine (Regitin® 5 mg/kg b wt)

In some experiments the intracutaneous temperature was checked by means of a thermoelement inserted into the upper lip During sympathetic stimulation a slight lowering of the skin temperature was observed probably as a consequence of a con comitant vasoconstriction Clamping of the ipsilateral common carotid artery gave a corresponding decrease in skin temperature but no changes whatever were seen in the responses to mechanical stimulation for time periods of up to three min Application of cervical sympathetic stimulation during a carotid occlusion on the other hand resulted in the same changes as those observed during intact circula tion

Discussion

The present investigation was prompted by the morphological finding that the sinus hairs in the cat contrary to other tactile structures in the skin have a rich adrenergic innervation (Fuxe and Nilsson 1965) The experimental results show that sym pathetic stimulation at frequencies within the physiological range induces a small but repeatable change preferably in the dynamic sensitivity of the vibrissae re ceptors *The observed increase in spike latency and blocking of spikes are probably* a consequence of an increase in threshold to mechanical stimuli The results support the assumption of a physiological sympathetic modulation of sensory input from the vibrissae receptors by activation of the adrenergic terminals within the hair fol licles

Sympathetic nerve fibers have also been observed in close vicinity to other types of mechanoreceptors postulated to be under sympathetic control Thus in the frog Fuxe and Nil son (1965 ild demonstrate adrenergic terminals in the skin layers

probably containing the tactile receptors studied by Loewenstein (1956) Loewenstein and Altamirano-Orrego (1956) showed that the Pacinian corpuscles were sensitive to adrenergic drugs and Santini (1969) found adrenergic fibers in the central core of these corpuscles In electron microscope studies Andres (1971) discovered a close contact between sympathetic fibers and sensory endings in the muscle spindle previously, Hunt (1960) and others had shown that the response pattern from these receptors can be modified by sympathetic stimulation

The inhibition of the dynamic responses from the vibrissae receptors described in the present study resembles in some respects the effect of sympathetic stimulation on muscle spindles observed by Hunt (1960) In both cases the change in the response pattern occurs after a delay of 10—25 s and the lowest effective stimulus rates are about the same It is possible that an accumulation of noradrenaline up to a certain local concentration is required to produce a change in impulse discharge The same explanation may apply to the finding that the delay is increased by a reduction in stimulus frequency The uncertain effects obtained by i v injections of noradrenaline may also be due to an insufficient local concentration

The main difference in sympathetic modulation between the vibrissae and the muscle spindles is that the inhibition of the muscle spindle response is preceded by a slight facilitation for 10—20 s (Hunt 1960) No corresponding facilitation of the vibrissae receptors was observed except in one case in which an intravenous injection of noradrenaline caused a slight increase in discharge frequency The experimental arrangement used in the present study may however have failed to detect very small and transient facilitations

The sympathetic stimulation was followed by a reduction in blood flow in the facial skin observed as a slight lowering of the skin temperature It does however not seem likely that this vasoconstriction *per se* should yield the effects recorded in the vibrissae receptors since clamping of the common carotid artery had no effect whatever on impulse discharges or on responses to sympathetic stimulation In his studies on muscle spindles Hunt (1960) also found the effects of sympathetic stimulation to be the same whether the muscle was devoid of circulation or not The importance of changes in blood supply has however been stressed by other authors (Eldred *et al* 1960 Calma and Kidd 1962 Paintal 1964) who consider the sympathetic effects on muscle spindles to be attributable at least to a large extent to a vasoconstriction Also as regards another type of receptors in the cat the pain receptors of the tooth it has recently been claimed that the changes in impulse activity on stimulation of cervical sympathetic fibers should be due to reduced microcirculation and diminished metabolic activity (Edwall and Scott 1971)

In the present investigation the lowering of the facial skin temperature during sympathetic stimulation did not exceed 1 C and it seems unlikely that this small variation should result in a significant receptor depression The sensitivity of the vibrissae receptors to temperature changes is not known but similar slowly adapting skin receptors in the cat are comparatively little affected by temperature changes of this small magnitude (Tapper 1965)

The vibrissae follicles are surrounded by striated muscles supplied by the facial nerve (Vincent 1913) No vibrissae movements were observed in the microscope during the period of sympathetic stimulation, and hence the changes in impulse responses cannot be attributed to mechanical factors such as contractions of pilomotor muscles Further the sympathetic modulation of the vibrissae receptors was independent of the direction of the mechanical stimulus and could be de monstrated also when the stimulator was used to push the hair inward Thus the modulation cannot be attributable to an induced change in the position of the vibrissae

Even though the experiments have established that sympathetic activity modifies the impulse responses it is still an open question whether this effect is achieved by the locally released noradrenaline acting directly on the receptor membrane or by this release *influencing in ome way the chemical or mechanical properties* of the immediate surroundings of the receptors *e g* by changing the microcirculation through the blood sinus of the follicle

The sensory information from the vibrissae receptors is of great importance for the cat s capability of orientation in the dark (Schmidberger 1932) Whether the slight modification that can be achieved by increased sympathetic output is of any functional significance in this connection is difficult to judge In view of the comparatively small effects of the sympathetic nerve stimulation the question arises whether the svmpathetic terminals in the hair follicles may serve some other purpose ond that of producing the excitability changes demonstrated in these experi nts

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Centrally Mediated Effects of Sodium and Angiotensin II on Arterial Blood Pressure and Fluid Balance

By

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Abstract

ANDERSSON B L ERIKSSON O FERNANDEZ C G KOLMODIN and R OLTNER *Centrally mediated effects of sodium and angiotensin II on arterial blood pressure and fluid balance* Acta physiol scand 1972 *85* 398—407

Four kinds of infusions were made for 1 h periods into the 3rd cerebral ventricle of hydrated conscious goats 1) Angiotensin in hypertonic NaCl 2) angiotensin in slightly hypotonic NaCl 3) angiotensin in isotonic d glucose and 4) hypertonic NaCl The influence of these infusions on carotid blood pressure and fluid balance was studied The infusions of angiotensin in hypertonic NaCl had conspicuous hypertensive dipsogenic antidiuretic and natriuretic effects More moderate responses of the same kinds were obtained during the infusions of hypertonic NaCl and of angiotensin in slightly hypotonic NaCl In contrast no or only weak effects were obtained by the infusions of angiotensin in isotonic d glucose The results are taken as support for the idea that a Na sensitive system in the vicinity of the 3rd ventricle may be of importance in the central control of fluid balance and arterial blood pressure Such a system may be influenced by the Na concentration of the cerebrospinal fluid and angiotensin may either sensitize it to Na or make it more exposed to Na ions

Infusions of hypertonic Na salt solutions into the cerebrospinal fluid (CSF) of the 3rd ventricle of the goat elicit thirst release of antidiuretic hormone (ADH) and natriuresis (*cf* Andersson and Olsson 1970) The same responses are obtained by intraventricular infusions of angiotensin II dissolved in iso or slightly hypotonic saline A potentiation of the dipsogenic antidiuretic and natriuretic effects is seen when this substance and hypertonic NaCl are infused together into the 3rd ventricle (Andersson and Eriksson 1971) On the other hand if angiotensin II (hereafter referred to as angiotensin) is administered in iso- or hypertonic solutions of non electrolytes its effect on central mechanisms controlling the fluid balance becomes markedly reduced (Andersson Erikson and Oltner 1970) It appears therefore

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that the effect of angiotensin on these brain mechanisms in some manner is mediated by Na ions

A preliminary report (Andersson Eriksson and Fernandez 1971) has been given of expts which indicate that also the centrally mediated hypertensive effect of angiotensin (*cf* Severs *et al* 1970) reflects central sodium—angiotensin interaction Here is presented a further analysis of the influence of the Na concentration of the CSF on arterial blood pressure and fluid balance in the goat

Methods

Animals and animal care 6 adult female goats (b wt 32 to 39 kg) were used The animals were routinely confined in metabolism cages and all expts were conducted in these cages The goats had *free access to chaffed hay and water at a temperature of 20±1 C* The were main tained in positive sodium balance by getting 6 g of NaCl in 400 g of commercial grain mix each afternoon

Implantations into the 3rd ventricle and infusion technique All goats were prepared with a permanent cannula in the anterior part of the 3rd cerebral ventricle The implantation and infusion techniques have been described earlier (Andersson Olsson and Warner 1967) In all expts CSF was observed to drain out of the permanent cannula on compression of the neck before insertion of and after removal of the inner cannula used for the infusions Thus free mixing of the infused solution with the CSF of the 3rd ventricle occurred in all expts

Induction of diabetes insipidus In addition to the ventricle cannula, one of the goats also had a pair of thermo-couple electrodes permanently implanted bilaterally in the median eminence region At a certain stage during the experimental period radio-frequenc heating was appl ed between the uninsulated tips of these electrodes The lesion interrupted the neural connection between the hypothalamus and the neurohypophysis and induced diabetes insipidus For further details about the technique see Olsson (1970)

Blood pressure recording 5 of the goats had a polyvinyl catheter permanently implanted via the superficial temporal artery into the carotid artery as described by Olsson and co-workers (Eriksson Fernandez and Olsson 1971) The catheters were flushed with heparin solution every second day During the expts the arterial catheter was connected to a Stratham pressure transducer Mean and systolic/diastolic blood pressures were recorded on an ink writing polygraph (Fig 3)

Intravenous injections and infusions Intravenous injections and infusions were made by use of a polyethylene cannula introduced into the jugular vein

Urine collection and analyses The urine as collected in 10 min samples via a retention catheter inserted into the urinary bladde Urine Na was determined by use of an EEL flame photometer A Knauer or an Advanced Instruments Inc osmometer was used for determinations of plasma and urine osmolality Since the mean pre infusion plasma osmolality was 290 mosm/ kg th s value was constantly u ed for calculation of renal free water clearance (C_{H_2O})

Planning of the experiments

In order to observe the antidiureti effect of th infus on and to obtain optimal cond tions for natriuresis (Andersson and Eriksson 1971) all infus ons into the 3rd entri le were pe formed in the pre hydrated goat The an mals we e g en 100 ml/kg b w t of 37 C wat by stomach tube into the rumen 70 to 90 min before ar infu on was started The expts we e grouped in series of 4 intraventricular nfusions perfo med with a minimum interval of days The in fusions in each serie consisted of

1) Angiotensin d ssolved in slightly hypotonic (0 14 M) NaCl solution
2) Angiotensin in otonic (0 3 M) d gluco e solution
3) Angiotensin in hypertonic NaCl solut on and
4) Merely hypertonic NaCl solution

The dose of angioten in nfused and the osmolal ty of the hyp rton NaCl solut on w maintained constant witl in each exp rimental series The amount of ang otensin gi 0 8 ng/kg min (5 series) and 0 4 ng/kg min (6 series) The hyperton c NaCl w (5 series) and 0 33 M (6 series) In all expts the duration of the ntraventricular i 1 h and the ate of infusion 10 µl/min

Hypertensin Ciba in 0 5 mg ampoules was used to prepare the angiotensin solutions In 2 of the expts involving the intraventricular infusion of angiotensin in isotonic d glucose the angiotensin was pre incubated in saline The 0 5 mg of angiotensin used to make up the final solution was dissolved in 2 ml of 0 15 M NaCl 2 h before it was added to 298 ml of 0 3 M d glucose

Results

1 Infusions into the 3rd ventricle

All the 4 parameters studied were strikingly correlated Conspicuous dipsogenic antidiuretic hypertensive and natriuretic responses were obtained by the combined infusion of angiotensin and hypertonic NaCl The responses to the infusions of angiotensin in 0 14 M NaCl or of merely the hypertonic NaCl were more moderate No or only weak effects were obtained by the infusion of angiotensin dissolved in isotonic d glucose regardless whether pre incubated angiotensin (see Methods) was used or not Each effect of the infusion is considered separately below

Thirst

In order to avoid over hydration no animal was allowed to drink more than 15 % of its initial water load during the infusion periods (< 500 ml) If this amount had been drunk during an infusion the water was with held and only negligible amounts were offered at intervals for some time after the infusion in order to determine the post infusion duration of the dipsogenic effect

In all 11 experimental series the combined infusion of *angiotensin and hypertonic NaCl* induced the goats to drink the total amount of water available The mean latency time for drinking was 3 1/2 min (range 1 1/2 to 8 min) Obvious thirst out lasted the infusion periods by 15 to 30 min During 9 of 11 infusions of *angiotensin in 0 14 M NaCl* the animals drank all the water available In 3 of these expts the goats were no longer thirsty at the termination of the infusion period If the animals were thirsty at this stage the urge to drink disappeared within the following 10 min The mean latency time for drinking was 11 min (range 2 1/2 to 30 min) One of the infusions of angiotensin (0 4 ng/kg min) in 0 14 M NaCl did not induce drinking All but one of the infusions of *hypertonic NaCl* induced drinking The total amount of water available was drunk during 9 of these infusions, but the thirst always disappeared within 5 min after the infusion The mean latency time for the dipsogenic response was 13 min (range 3 to 49 min) during the effective infusions of hypertonic saline

None of the goats drank during the infusion of *angiotensin in isotonic d glucose* However in 3 of the expts (when the larger dose of angiotensin had been infused) the goats sipped small amounts of water about 5 min after termination of the infusion

Inhibition of the water diuresis

In the pre hydrated goats the water diuresis was fully developed (renal C_{H_2O} about 5 ml/min) at the start of the infusion The renal C_{H_2O} became negative during all infusions of hypertonic NaCl and of angiotensin in hypertonic or 0 14 M NaCl (Fig

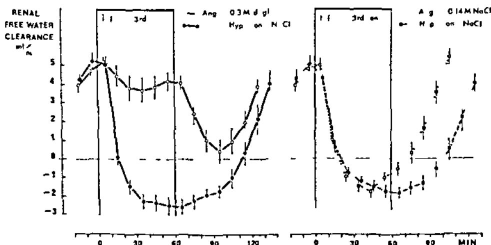


Fig 1 *An illustration of central sodium—angiotensin interaction on the release of ADH in the hydrated goat* Mean (±SEM) of 11 experimental series each including all 4 kinds of *infusions into the 3rd ventricle Dose of angiotensin II = 0 8 ng/kg min (5 series) and* 0 4 ng/kg min (6 series) Hypertonic NaCl = 0 25 M (5 series) and 0 33 M (6 series) Rate of infusion = 10 μl/min Note that the main antidiuretic effect of angiotensin in isotonic d glucose appears *after* the infusion

1) The strongest antidiuretic effect was obtained by the infusion of *angiotensin in hypertonic NaCl* The renal C_{H_2O} reached its lowest level (range −1 7 to −4 4 ml/min) at the end of the infusion period and remained negative for about 1 h after the infusion Also during the infusion of merely the *hypertonic NaCl* the renal C_{H_2O} reached its minimum value (range −1 to −3 5 ml/min) at the termination of the infusion and remained negative for more than 30 min after the infusion The antidiuresis seen as effect of the infusion of *angiotensin in 0 14 M NaCl* was of shorter duration When the smaller dose of angiotensin was administered the renal C_{H_2O} started to return towards zero even before the infusion was finished

No or only a weak antidiuretic response was obtained *during* the intraventricular infusions of *angiotensin in isotonic d glucose* A transient fall in renal C_{H_2O} was seen during 4 infusions when the larger dose of angiotensin was given During 2 of these infusions the renal C_{H_2O} fell to zero but became positive again before the infusion was finished In the other 7 expts the renal C_{H_2O} remained at the high pre infusion level throughout the entire infusion period However a distinct post infusion inhibition of the water diuresis was seen after all but one of the infusions of angiotensin in isotonic d glucose (Fig 1 *left*)

The antidiuresis which results from infusions of hypertonic NaCl into the 3rd ventricle by all probability is due to release of ADH from the neurohypophysis since it is no longer seen after the induction of diabetes insipidus (Andersson Dallman and Olsson 1969 a) This apparently also holds true for the antidiuretic respons infusions of angiotensin into the 3rd ventricle After completion of two exp series diabetes insipidus was induced in the goat having thermo-coupl

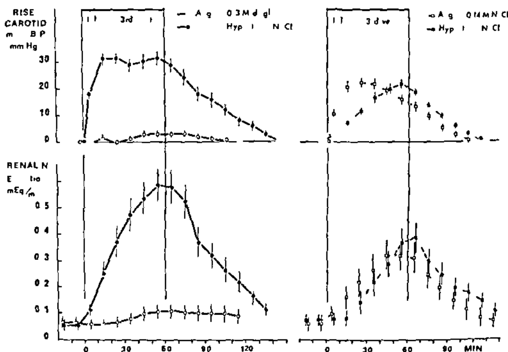


Fig 2 A comparison between the hypertensive and the natriuretic responses in the hydrated goat to intraventricular infusions of angiotensin in 3 different solutions and of merely hypertonic NaCl Mean (±SEM) of 9 experimental series each including all four kinds of infusions into the 3rd ventricle Dose of angiotensin II = 0 8 ng/kg min (4 series) and 0 4 ng/kg min (5 series) Hypertonic NaCl = 0 25 M (4 series) and 0 33 M (5 series) Rate of infusion = 10 µl/min

implanted bilaterally in the median eminence region Intraventricular infusion of angiotensin (0 8 ng/kg min) in 0 33 M NaCl were made for 30 min and 1 h as well during the transient as during the permanent stage of diabetes insipidus in this animal No inhibition of the water diuresis was obtained in these expts

Hypertensive and natriuretic effects

The pre infusion carotid mean blood pressure was within the range of 80 to 90 mm Hg in the 5 goats used to study the blood pressure response to infusions into the 3rd ventricle The blood pressure was recorded in 9 experimental series A pronounced and long lasting rise in the carotid mean pressure was obtained by all infusions of angiotensin in hypertonic NaCl In contrast no or only slight blood pressure increase occurred during the infusion of angiotensin in isotonic d glucose The infusions of merely hypertonic NaCl or of angiotensin in 0 14 M NaCl had intermediate pressor effects (Fig 2 above)

During the infusions of *angiotensin in hypertonic NaCl* the blood pressure started to rise within 5 min and reached its maximum (range of increase 25 to 45 mm Hg) 5 to 20 min later It remained high throughout the infusion period and then slowly declined The pressure did not return to pre infusion level until 50 to 90

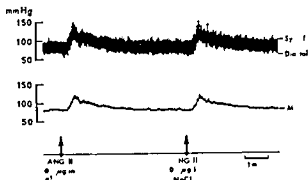


Fig 3 Pressor effects of two consecutive intravenous injections of angiotensin II in 0.3 M d glucose and in 0.15 M NaCl Carotid blood pressure recorded in a concious goat (b wt. 36 kg) Dose of angiotensin = 0.4 μg in a volume of 0.1 ml

min after cessation of the infusion Also during the infusion of *angiotensin in 0.14 M NaCl* the blood pressure reached its maximum (range of increase 15 to 35 mm Hg) during the first half of the infusion period However, when the smaller dose of angiotensin administered the blood pressure started to decline before the infusion was finished Return to pre infusion blood pressure level occurred 20 to 50 min after the infusion

The carotid pressure rose more slowly during the infusions of *hypertonic NaCl* The highest blood pressure level (range of increase 15 to 30 mm Hg) was not reached until the end of the infusion period The decline to pre infusion blood pressure took place within 40 to 60 min after the infusions were stopped Four of the 9 infusions of *angiotensin in isotonic d glucose* had no measurable effect on the carotid blood pressure In the remaining 5 expts a slight rise (5 to 10 mm Hg) was observed towards the end of and within 30 min after the infusion period

Although great variations were seen between animals the magnitude and the duration of the natriuretic responses to the different infusions generally were correlated to the same parameters for the hypertensive responses (Fig 2 *below*)

B Intravenous infusions and injections

Angiotensin

Infusions Intravenous control infusions of angiotensin dissolved in isotonic saline were made for 60 min periods in two of the goats during hydration The amount of angiotensin infused was equal to the larger dose earlier administered into the 3rd ventricle in these animals (0.8 ng/kg min) The infusions caused no observable increase in carotid mean pressure and had no dipsogenic antidiuretic or natriuretic effects

Injections For reasons to be discussed below it was of interest to study whether the intravenous injection of angiotensin in isotonic saline would have a stronger pressor effect than injections of the substance dissolved in isotonic d glucose Repeated tests were made in two of the goats with solutions prepared 1 h before the first injection was made The amount of angiotensin injected each time was 0.4 in a volume of 0.1 ml of either isotonic NaCl or d-glucose The injections

a marked transient rise in carotid blood pressure (Fig 3) As shown in the Table below there was no significant difference in the pressor effect of the two solutions

Intravenous inj	*Carotid B P* Mean rise mm Hg ± SEM	*n*
Angiotensin in NaCl	45 4 ± 1 7	18
Angiotensin in glucose	45 2 ± 1 5	18
t = 0 099	p > 30 %	

Antidiuretic hormone (ADH)

Intravenous infusions of ADH (Arginine vasopressin Sigma 0 1 m U/kg min) were made for 30 min periods in two of the goats during hydration As expected these infusions had a pronounced and sustained antidiuretic effect However, the infusion of this amount of ADH did not cause any rise in carotid blood pressure or in the renal Na excretion

Discussion

The expts reported here confirm two observations earlier made in the same species 1) An experimentally induced elevation of the Na concentration of the CSF in the 3rd ventricle influences the water balance in positive and the salt balance in negative direction 2) The local Na concentration determines to which extent in fusions of angiotensin into the 3rd ventricle affect the fluid balance of the animals In addition to this the present expts show that also the hypertensive response to angiotensin administered into the 3rd ventricle (*cf* Severs *et al* 1970) is dependent upon the local Na concentration and that an elevation of ventricular Na concentration in itself may cause a rise in the arterial blood pressure A crucial question obviously is whether it may be justified to interpret these experimental observations in terms of the physiological control of body fluid homeostasis and blood pressure

The current idea is that osmometric and volumetric mechanisms regulate the urge to drink and the release of water saving ADH Much evidence has been produced that the water balance principally is controlled by these two regulations which appear complementary to each other in a common endeavour to maintain stability in the composition and the volume of the body fluids Verney s fundamental studies (Verney 1947 Jewell and Verney 1957) have made the idea widely accepted that hypothalamic osmoreceptors (sensitive to changes in their own volume) are essential for an osmometric regulation of ADH release and thirst

The kidney appears to be of importance in a volumetric control of the water balance It was originally shown by Linazasoro and co workers that removal of the kidneys reduces the water intake in rats and that drinking under such circumstances may be restored by the injection of extracts of kidney tissue (Linazasoro Jimenez Dias and Castro Mendoza 1954 Jimenez Dias Linazasoro and Castro Mendoza 1959) The importance of a renal factor in the volumetric regulation of water intake has been further elucidated by Fitzsimons He has shown that hypovolemic activation of the renin angiotensin system stimulates the thirst mechanism in the

rat (Fitzsimons 1961, 1969) and that this most likely is due to an action of angiotensin at the hypothalamic level of the brain (Epstein Fitzsimons and Simons 1969) Angiotensin also enhances the release of ADH from the neurohypophysis (Bonjour and Malvin 1970) apparently by affecting the cerebral control of the liberation of this hormone (Andersson *et al* 1970)

The main evidence for the existence of hypothalamic osmoreceptors and for an osmometric control of the water balance has been obtained by studying the effects of alterations in the solute composition of the blood plasma The possible interaction of brain barrier systems appears to have received too little attention in these studies When the blood brain barrier is taken into consideration some aspects of the osmoreceptor theory may be criticized A rather effective blood brain barrier exists for urea glycerol and sodium ions (Yudilevich and de Rose 1971) Therefore their intravascular application in equi osmolal hypertonic solutions is likely to cause the same degree of brain dehydration and to act as equivalent stimuli to osmoreceptors located inside the blood brain barrier Nevertheless a rise in the carotid blood osmolality obtained by infusions of hypertonic urea and glycerol acts as a much weaker stimulus to ADH release and thirst than the same rise in blood osmolality elicited by the intracarotid infusion of hypertonic NaCl (Eriksson *et al* 1971 Olsson 1972) The existence of osmoreceptors inside the blood brain barrier can be called in question also for another reason The infusion of hypertonic sucrose into the 3rd ventricle of the goat does not elicit thirst or release of ADH (Olsson 1969), in spite of the fact that the sucrose molecules do not pass into the cells This makes it unlikely that the polydipsic and antidiuretic effects which are seen during infusions of hypertonic NaCl into the 3rd ventricle reflect osmoreceptor stimulation On these grounds it has recently been suggested (Andersson 1971) that a possible alternative to hypothalamic osmoreceptors in Verney's (1947) sense would be receptors which are situated near the 3rd ventricle and which are influenced by the Na concentration of the CSF An explanation for the sodium angiotensin interaction observed here and in previous studies in the goat may be that angiotensin either makes such Na receptors more sensitive to the environmental Na concentration or in some manner makes the receptors more exposed to Na ions Therefore, a volumetric regulation of thirst and ADH release which is mediated by the renin angiotensin system also may have its final link in such Na receptors The experiments reported here indicate that a Na sensitive system of similar kind and location also may play a role in the regulation of the arterial blood pressure

Recent investigations in other species lend some support to the ideas expressed above Studies of the exchange of ^{24}Na between blood and brain in the rhesus monkey have revealed that sodium ions in the blood diffuse in much higher amounts in the CSF and the brain tissue bordering the ventricles than in other parts of the brain (Milhorat *et al* 1971) A condition precedent for a physiological [illegible] cation of the observed sodium angiotensin interaction is obviously that angiotensin or its precursor is transferred from the blood to the CSF, or to the surroundings of the 3rd ventricle Studies of the distribution of labelled angiotensin in [illegible]

intravenous injection in the mice show that angiotensin enters the CSF via the choroidal plexus of the lateral ventricles and appears inside and around the 3rd ventricle (Volicer and Loew 1971) Of particular interest is also that a renin like angiotensin forming enzyme is present in the brain tissue of dogs and is independent of kidney and plasma renin Administration of the sodium retaining hormone aldosterone significantly decreases this angiotensin forming enzyme activity (Ganten *et al* 1971)

It was recently observed in the rat and guinea pig that the intravenous injection of angiotensin in aqueous solution has less pressor effect than similar injections of angiotensin in iso- or hypertonic saline (Bergmann *et al* 1971) It has led to the suggestion that Na may complex with angiotensin to form a more active molecule and that this could explain the central sodium angiotensin synergism observed in the goat The explanation appears unlikely for several reasons The intraventricular infusions of angiotensin in d glucose had very weak effects whether or not NaCl incubated angiotensin was used to make up the final solution The intravenous injection of angiotensin in d glucose caused the same rise in carotid blood pressure as the injection of the substance dissolved in isotonic NaCl (Fig 3) In most expts the antidiuretic and hypertensive effects became reduced towards the end of the intraventricular infusions of angiotensin in 0 14 M NaCl This can not have been due to a reduction in the potency of the angiotensin molecules as such A more likely explanation appears to be that the ADH induced water retention gradually lowered Na concentration of the CSF and hereby reduced the sodium angiotensin interaction The opposite may explain the antidiuretic effect generally seen after the intraventricular infusions of angiotensin in d glucose (Fig 1 *left*) Angiotensin may have remained in or near the 3rd ventricle in amounts sufficient to interact with Na when glucose no longer reduced the Na concentration of the ventricular fluid

Studies in the rat have shown that the pressor effect of angiotensin administered into the ventricular system is the result of both increased vascular sympathetic tone and vasopressin (ADH) release (Severs *et al* 1970) Further expts are needed to reveal whether the hypertensive responses obtained in this study also had this dual cause ADH release was obviously the cause of the antidiuresis since the response was blocked by the interruption of the connection between the hypothalamus and the neurohypophysis However the intravenous control infusions of ADH show that water retention during the antidiuresis does not increase the carotid blood pressure significantly If ADH did contribute to the hypertensive response in the present expts the hormone must have been released in an amount exceeding that given during the intravenous control infusions

It has previously been shown that the natriuresis which is induced by infusions of hypertonic NaCl into the 3rd ventricle of the goat is accompanied by an increase in the glomerular filtration rate (Andersson Dallman Olsson 1969 b) The observed correlation between the hypertensive and natriuretic effects (Fig 2) also indicates that changes in renal hemodynamics may have contributed to the natriuretic response

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Acta physiol scand 1972 85 408—413
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Single Glomerular Blood Flow as Measured with Carbonized 141—Ce Labelled Microspheres

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Abstract

KÄLLSKOG Ö H R ULFENDAHL and M WOLGAST *Single glomerular blood flow as measured with carbonized 141 Ce labelled microspheres* Acta physiol scand 1972 *85* 408—413

The blood flow to single cortical and juxtamedullary glomeruli was investigated in "normal rats by means of a microsphere technique Radioactively labelled microspheres were injected retrogradely into the ascending aorta and the number of spheres trapped in the glomeruli at different levels of the cortical parenchyma was determined by gamma spectrometry and related to the number collected from a catheter in the femoral artery A uniform sphere size and no aggregates in the injected bolus were essential requirements for accurate blood flow determinations The spheres were separated by sedimentation in water which reduced the size range from a nominal value of 15±5 μ to 16 9±2 65 μ and gave no aggregates in the sedimented sample The single glomerular perfusion was estimated to be 0 23 μl/min and 0 20 μl/min for cortical and juxtamedullary glomeruli respectively The difference was not significant (P > 0 5 The values correspond to estimations of total renal blood flow in the present and previous investigations

It is now established that the distribution of the blood flow in the kidney is in homogeneous with a very high cortical perfusion amounting to 4—5 ml/min and gram tissue whereas in the medulla there is a progressive decrease from about 1 5 ml/min in the outer zone to about 0 5 ml/min and gram tissue in the inner part of the inner zone (Ullrich *et al* 1961 Wolgast 1968) Concerning the intracortical blood flow distribution opinions are divergent Studies in which the clearance of inert diffusible indicators have been used as an index of the regional blood flow have shown an uneven distribution under control conditions (Thorburn *et al* 1963) as well as in conditions of hemorrhagic shock (Carrier *et al* 1966) Aukland (1964) found in contrast a more or less homogeneously distributed cortical blood flow on measuring the wash-out rate of hydrogen from the parenchyma Recent studies of the regional cortical blood flow using the microsphere technique (McNay and Abe 1970) indicate a comparatively low blood flow in the innermost layer of the cortical parenchyma

In recent years the regulation of the blood flow to the cortical and juxtamedullary glomeruli has been the object of several investigations (Horster and Thurau 1968). It has only been possible however to obtain a rough estimate of the single glomerular perfusion from filtration data and no method has been developed permitting measurement of the blood flow in individual glomeruli. In the present investigation the microsphere technique has been applied to measurement of the blood flow through single vascular units i.e. the single glomerular flow.

Methods

The experiments were carried out on 10 male rats (Sprague/Dawley weighing bet een 200 and 300 g. Anesthesia was induced by intraperitoneal administration of Inactin® in a dose of 100 mg/kg (Chem Fabrik Promonta GmbH Hamburg West Germany). The animals were then tracheotomized and placed on a servo-controlled heating pad. For the microsphere injection a 1 mm thick polyethylene catheter was introduced into the right carotid artery and advanced until its tip was located just above the aortic valves. Blood pressure recording and blood sampling were performed via a catheter inserted in the femoral artery.

The microspheres (^{141}Ce labelled 3 M Co St Paul Min U S A) were first treated with ultrasonic agitation and then separated by s dimentation in water to which one droplet of a detergent (Tween 20) had been added. The process had to be carried out in relatively large tubes about 6 cm in diameter in order to avoid adherence of the spheres to the tube walls. After 20 min the particles had spread over a distance of some 30 cm. The middle about 10 cm long fraction was then used for the inject ons. The size of the spheres thus obtained was estimated to be 16.9 ± 2.65 μ (mean ± S D). This value may be compared with the figure of 15 ± 5 μ given by the manufacturers for the original batch—a figure which also was found in our own investigations. The greatest advantage of the separation procedure was however that aggregates present in the original batch could be totally eliminated. The spheres were then suspended in rat plasma as plasma suspensions were found to be more stable than saline suspensions.

The experiments were conducted as follows: 0.5 ml of the suspension was injected in 5 s. At the same time arterial blood was collected from the femoral catheter by free flow. The collecting time was 1 min during which period about 1 ml of blood was obtained. The flow rate did not change significantly during the collection per od nor did the blood pressure.

The rat was then killed and the kidneys remo ed and placed in a 50 hydrochloric acid solution at 40 C for 1 h for maceration. P eces of the cort al pa enchyma were then divided into 2 slices the first constituting the one mm thick nnermo t layer of the parenchyma and the second the outer parts: the first layer contained the so called juxtamedullary glomeruli and the second mainly purely co tical glomeruli. The slices were then suspended in isotonic saline. 50 glomeruli from each su pension were then p cked up w th a glass capillary with the guidance of a microscope with 100 times magnification and placed on glass f ber discs (Whatman glass fiber paper W o R Balston Great Britain) and counted in a gamma spectrometer (Intertechnique multi channel analyzer mod Didac 800) for 10 min. The blood samples ere analysed in the same way.

During the collection of the glomeruli the number of phe es lo ated in each glom rulus was counted.

Calculation

Assuming that the micro pheres w ll be evenly mix d w th the blood at the inj ction site and that they will be dist ibuted proportionately to the blood flow to the d fferent tis ues where they will be trapped in the capillary system, the blood flow to the area und r stud f will be governed by the equation f m F/M where m is the amount of spheres t apped M the total amount injected F the blood flow at the site of injection of the sphere i.e. n th ase equal to the cardiac output. The flow then refers to cap llary flow. Blood flowing through a v shunts in which no spheres are t app d could obv ously not be record d.

The amount of sph eres collected in the sampled blood will al o be proportional to the flow of blood thro igh the ollection catheter i the collection rat. The blood flow to the tissue region in question f can then be related to the sampling rate according to the equation f=m f /m where f is the sampling rate and m the amount of spheres found in the sampled blood. The latter method has in fact great advantages over the former calculation

TABLE I Summary of the blood flow data from 10 rats The values are given in mean ± S D

Arterial pressure mm Hg	Glomerular blood flow µl/min Cortical	Juxtamedullary	Blood flow ratio Cortical/ Juxtamedullary	Ratio between the number of spoheres trapped Cortical/ Juxtamedullary	Total blood flow of the investigated kidney ml/min per kg rat
108 4 ± 8 7	0 23 ± 0 09	0 20 ± 0 12	1 2 ± 0 3	1 2 ± 0 1	21 ± 7

any recirculating particles will lead to additional trapping of microspheres and thus result in an overestimation of the blood flow when the first calculation is used This source of error is, however not important since the spheres escaping from the microcirculation will be trapped to a large extent in the pulmonary circulation Secondly from a technical point of view the *latter method is simpler and eliminates the difficulties in the amount injected* which has proved to be a problem with the spheres used

Results

By the maceration technique used, the structure of the kidney was maintained which permitted localization of microspheres trapped in large vessels The different structural elements could however be easily separated from each other and suspended in saline The vascular walls were not destroyed and the glomeruli were intact The spheres were found to be almost exclusively *located to the glomerular capillary net*work and very few were observed in the afferent arterioles and in the interlobular arteries In earlier experiments when non separated spheres were used large spheres and especially aggregates of spheres were found to some extent in the interlobular vessels The amount of recirculating microspheres was checked by analysing the amount trapped in the lungs The results indicated that about 1 % or fewer had escaped from the microcirculation during their first transit

The results from determinations of the blood flow to single cortical and juxtamedullary glomeruli and of the total blood flow of the investigated kidney are presented in Table I The amount of blood perfusing the cortical glomeruli was estimated to be 0 23 ± 0 09 µl/min and that perfusing the juxtamedullary glomeruli 0 20 ± 0 12 µl/min The difference between the blood perfusion rate in cortical and juxtamedullary glomeruli was not statistically significant (P > 0 5) Assuming that the number of glomeruli per rat kidney is in the order of 30 000 the total renal blood flow of one kidney was calculated to be 6 6 ml/min This value agrees essentially with the total renal blood flow of 5 6 ml/min as calculated from the whole kidney activity found in this series

In order to determine whether spheres of different sizes were trapped in different areas which would then give an error in the blood flow estimations as calculated from the radioactive analyses the actual number of spheres in the glomeruli were counted The ratio between the number of spheres in cortical and juxtamedullary glomeruli was then found to be 1 2 ± 0 1 This value is not statistically different from the calculated blood flow ratio of 1 3 ± 0 3 (P > 0 5)

Discussion

The microsphere method is based on the assumptions that the spheres will mix completely with the blood at the injection site and that they will behave as blood constituents during their passage through the main arteries and that they will be trapped quantitatively in the microcirculation

The problem of complete mixing has been largely solved by retrograde injection into the ascending aorta near to the aortic valves This injection will probably lead to complete mixing with the blood leaving the aorta there may however be incomplete mixing with the blood perfusing the coronary arteries Even though the spheres used have specific gravity of about 1 3 there is reason to believe that they do behave as blood constituents Phibbs *et al* (1968) on rapid freezing of medium sized artery found no evidence of sedimentation of the particles and concluded that the flow distribution of the spheres was similar to the blood flow distribution from a medium sized artery to a smaller branch This method has been compared with the Rb^{86} extraction method (see Sapierstein 1956 and for criticism Wolgast 1968) in the hindleg with sarcoma (Lewis 1968) No difference in distribution between the two indicators was found in muscle skin and tumor In the present investigation this question is very pertinent since the vascular arrangement of the cortical circulatory system means that skimming effects might be expected *i e* that large and small particles may be distributed in a different manner to deep and superficial glomeruli (Pappenheimer and Kinter 1956) The good agreement between the ratio of the blood flows to deep and superficial glomeruli and the ratio of the visually counted number of spheres trapped speaks however against the possibility that large and small particles are distributed in a different manner

It may be pointed out in this connection that the proposed skimming of blood in the cortical vascular tree does not seem to be very important under normal conditions as found by Ulfendahl (1962) in determining the hematocrit in blood from the deep venous system in relation to the superficial cortical drainage The total renal blood flow as calculated on both kidneys per kg b w was estimated to be 42 ml/min In previous investigations by several authors (see Smith 1951) the PAH clearance (effective plasma flow) has been found to be about 20 ml/min per kg/b w which means a total blood flow of about 50 ml/min *i e* of the same order of magnitude as in the present investigation

It must be stressed that a prerequisite for a valid comparison between total renal blood flow and the single glomerular perfusion rate multiplied by an assumed number of glomeruli is that no spheres are trapped in the large vessels in the renal parenchyma In earlier experiments where non separated particles were used the total renal blood flow of the investigated kidney was much higher when estimated from analyses on the whole parenchyma than when calculated from the single glomerular perfusion rate This was consistent with the finding that large spheres and especially aggregates of spheres were trapped in large vessels—the interlobular arteries and even in the arcuate arteries and interlobar arteries In attempts to obtain valid data on the blood flow to very small areas as in this case

glomeruli the size of the spheres used and the size range is very important For determinations of the total renal blood flow it is only necessary in principle to have particles with a size range from that of say, the arcuate arteries to the size of the small peritubular capillaries but when estimating the glomerular perfusion rate we consider that the spheres should be trapped only in the glomerular network and that trapping in afferent arteriolae will obviously disturb the glomerular circulation and prevent any additional trapping in the glomeruli This is essential since measurement of the blood flow to single small structures such as glomeruli requires a relatively large number of spheres in the injected bolus which in this series is restricted to a number giving one or less spheres per glomerulus Pilot experiments in which 2—4 spheres were trapped in the glomeruli did not seem however to affect the systemic circulation as judged by the blood pressure and total renal blood flow

The data obtained have the dimension of blood flow per vascular unit The values cannot be related directly to regional blood flow values expressed in ml/min and g tissue not even qualitatively since in the latter case the blood flow values obtained can change with alterations in the volume of the organ which certainly will be of importance where the kidney is concerned The present method is unaffected by these alterations however The blood flow in the medullary parenchyma cannot be estimated with the technique used It could be argued that the medullary blood flow related to the juxtamedullary blood flow but as the absolute blood flow to the edulla constitutes only a part of the blood perfusing the juxtamedullary glomeruli (see Moffat 1967) the values obtained will not give the absolute medullary blood flow

We consider that determination of blood flow per vascular unit is a fruitful measure in estimating the role of the circulatory system in the renal function The technique is simple but requires careful handling of the spheres including separation of individual spheres into narrower fractions It can be used in rats which means that the blood flow data can be compared with data for single tubules obtained by micropuncture techniques

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Acta physiol scand 1972 85 414—417

From the Institute of Physiology University of Helsinki Finland

Vagal and Sympathetic Efferent Discharge in the Bainbridge Reflex of Dogs

By

MARTTI O K HAKUMÄKI

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Abstract

HAKUMÄKI M O K *Vagal and sympathetic efferent discharge in the Bainbridge reflex of dogs* Acta physiol scand 1972 *85* 414—417

A study has been made of the response in vagal and sympathetic cardiac efferentation to i v injection in Bainbridge type experiments on dogs anesthetized with i v chloralose Cardiac vagal efferent activity was recorded from the cervical vagus and sympathetic cardiac efferentation from the postganglionic cardiac fibres An increase in sympathetic cardiac discharge along with diminution in the vagal cardiac discharge with associated tachycardia has been detected in the Bainbridge reflex of dogs A positive linear correlation was observable between the heart rate and the ratio of sympathetic and vagal number of impulses The acid base alance of the experimental animal was controlled

Bainbridge (1915) has demonstrated a cardiac acceleration response in dogs on the injection of i v saline or blood and suggested that an increase in sympathetic cardiac efferentation induced the reflex observed Marguth Raule and Schaefer (1951) and Jewett (1964) have found an antagonistic function between the sympathetic and vagal efferentation in different hemodynamic conditions Bergstrom Hakumäki and Sarajas (1971) have shown the existence of a positive correlation between the heart rate and the number of sympathetic efferent cardiac impulses in the Bainbridge reflex within a given time unit Warner and Russel (1969) have detected the dependence upon the stimulation parameters of the response of the heart rate to the simultaneous stimulus of vagal and sympathetic cardiac nerves Katona *et al* (1970) have demonstrated an increase in vagal efferent activity in the carotid body reflex The aim of this work is demonstration and quantitation of the interrelations of the heart rate and the sympathetic and vagal cardiac efferentation in dogs in which the Bainbridge reflex had been induced by i v injections of saline

Methods

The experiments were pe[illegible]rmed upon 8 young m[illegible]ngrel dogs of both sexes weighing 8 [illegible]—11 6 kg Th[illegible] dogs were [illegible]sed with morphine hydrochloride and anesthetized [illegible]ith i v chloralose Positive pres[illegible]tilation was applied by means of a Palmer respirator with different mixtures of 100 [illegible]en and room air according to the PO_2 values of arterial blood Thoracotomy was carried [illegible] the left side [illegible]f the thorax the sympathetic efferent activity was recorded from the cra[illegible] [illegible] of the left dorsal cervical cardiac nerve and the vagal

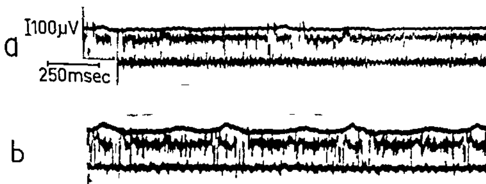


Fig 1 Heart rate and sympathetic postganglionic cardiac and vagal cardiac efferentation in a dog (10.3 kg) before (a) and 30 s after the injection of 170 ml of saline within 25 s (b) A rise in the *sympathetic discharge of impulses (the upper electroneurogram) per time unit* and a diminution in the vagal efferent discharge of impulses (the lower electroneurogram) per time unit is associated with cardiac acceleration The upper solid line indicates left atrial pressure

efferent activity (Type I Jewett 1964) from the left cervical vagus nerve The left femoral vein was cannulated for the injection of saline A cannula was also inserted into the femoral artery or the left atrium for pressure measurements When the nature of the Bainbridge reflex from cycle to cycle was observed before during and after the injection the number of efferent imp/s was calculated from one heart cycle and the heart rate determined with 3 cycles one before and one after the cycle in which the action potentials were calculated (*vide* Fig 2) For quantitation between the heart rate and the number of efferent impulses and their ratio the mean number of imp/s was calculated from successive samples recorded at intervals of 4 s before during and after the injection The heart rate was determined from a sample of the same duration but beginning 2 s after the point at which the calculation of action potentials had been started (*vide* Fig 3) A calculation of this kind was employed in view of the delay of 1—2 s between efferent stimulation and the response in the heart rate (Warner and Russel 1969) The amount of *saline injected into the left femoral vein varied between 80—200 ml* during a period of 20—60 s

The temperature of the fluids injected was kept equal to that of the left atrial blood (36.5—38° C) and tested with an electric thermometer The acid base balance was measured at intervals of 1/2 h by application of the Astrup technique the samples were taken from left femoral artery or atrial blood A solution of 7% sodium bicarbonate was injected into the femoral vein when needed under acidotic conditions

Results

In each of the 8 experimental animals there occurred an increase in sympathetic activity and a diminution in vagal activity during and after the injection A typical response in efferentation and heart rate is illustrated in Fig 1 and 2 The heart rate ranged from 54 to 100/min before injections during and after infusions it increased to 100—160/min The number of vagal efferent impulses varied between 14—50/s and the number of sympathetic postganglionic efferent impulses between 18—77/s in the control before injections during and after infusion the vagal activity declined to 5—38 imp/s and the sympathetic activity increased to 30—80 imp/s Apparently a positive inverse correlation exists between the heart rate and the number of vagal impulses The correlation coefficient varied from —0.554 to —0.984 (mean —0.792) and the number of points from 9 to 14 Moreover a positive correlation was observable between the heart rate and the number of sympathetic impulses The correlation coefficient varied from 0.491 to 0.974 (me

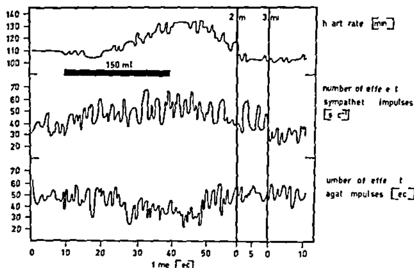


Fig 2 Influence of i v injection upon the heart rate/min (upper line) upon the number of sympathetic postganglionic efferent cardiac imp/s (lower line) and upon the number of vagal cardiac efferent imp/s (lowest line) before during and after injection calculated from cycle to cycle The upright 2 lines with marks of 2 min and 3 min illustrate pauses in the recording The heart rate was determined from 3 successive cycles and the number of imp/s from 1 cycle The figure illustrates an increase in the sympathetic efferent activity and a diminution in the vagal efferent activity followed by a rise in heart rate

948) and the number of points from 9 to 14 Since the sympathetic efferentation increases the heart rate and the vagal efferentation reduces it the ratio of the number of sympathetic and vagal impulses was calculated and compared with the heart rate (Fig 3) The correlation coefficient varied from 0 926 to 0 994 (mean 0 953) and the number of points from 9 to 14 It is obvious that a linear correlation exists between the heart rate and the ratio of the numbers of sympathetic and vagal impulses

The results obtained in the acid base analyses before the injections were as follows pH 7 38—7 42 PCO 38—42 PO 138—183

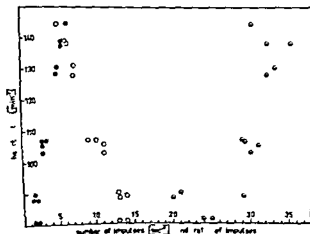


Fig 3 Heart rate/min as a function of the numbers of vagal efferent cardiac imp/s (open circles) numbers of sympathetic efferent postganglionic imp/s (half circles) and the ratio the numbers of sympathetic and vagal imp/s (closed circles) Each point represents a mean of the results of calculation in respect of s ce e periods f 4 s before during and after the injection

Discussion

In view of the results obtained earlier (Harry *et al* 1971, Ledsome and Linden 1964) the experiments were performed on dogs under controlled and normal acid base balance conditions to elicit the Bainbridge reflex with simultaneous recording of sympathetic and vagal cardiac efferent activity

The results arrived at in the present work demonstrate a reduction in cardiac vagal efferent activity and an increase in sympathetic cardiac efferentation with simultaneous increase in the heart rate These findings support previous reports of the existence of a certain area in the cardiovascular system possibly in the atria (Ledsome and Linden 1964 1967 Karim *et al* 1971), stimulation of which increases the sympathetic cardiac efferent activity and the heart rate

A linear correlation seems to exist between the heart rate and the ratio of sympathetic and vagal number of impulses This implies that the heart rate is not regulated by sympathetic or vagal efferentation alone but that the changes represent a result of simultaneous functioning of the two opposite systems It follows from this that both inhibitory and excitatory factors in the afferent and efferent systems need to be measured in the study of information transfer (Hakumaki 1970 1971) in cardiovascular regulation

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Effects of Alterations in Calcium Concentrations on Secretion and Protein Synthesis in Cat Submandibular Salivary Gland

By

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Abstract

Gautvik K M M Kriz and K Lund Larsen *Effects of alterations in calcium concentrations on secretion and protein synthesis in cat submandibular salivary gland* Acta physiol scand 1972 *85* 418—427

The effects of low Ca concentrations on secretory responses induced by injections of noradrenaline to the perfused cat submandibular salivary gland have been investigated Particular attention has been paid to the release of kininogenase (a kinin forming enzyme) Kininogenase activity was measured in saliva in effluent perfusate and in gland homogenate When perfusion media containing no added Ca was used a reduced amount of saliva with a normal concentration of kininogenase was secreted Additional reduction to near zero of [Ca] in perfusion fluid by the use of EGTA blocked saliva secretion However a fall in remaining kininogenase activity was still observed in homogenates from activated glands although less marked than in the activated control glands Low perfusate [Ca] also reduced the incorporation of C L-Leucine into proteins of non activated glands by an average of 52 per cent It is concluded that the secretion of water and ions is blocked and that the release and probably also the synthesis of glandular kininogenase is reduced under conditions of low concentration of Ca in the perfusate

The secretion of saliva from the submandibular salivary gland in cats can be elicited by stimulation of parasympathetic and sympathetic nerves The transmitters released from the postganglionic nerve terminals act on the gland cells whereby secretion is evoked The various factors linking the action of transmitters on gland cells to the ultimate secretory response are poorly understood From a series of studies Douglas (1963) has focused interest on extracellular Ca as an important factor in the general coupling between stimulus and the ultimate secretory response In their work on the perfused cat submandibular salivary gland Douglas and Poisner (1963) showed that secretion of saliva was profoundly reduced or abolished when calcium ions were omitted from the perfusion fluid

The release of organic substances *e g* enzymes into the saliva represents one interesting aspect of salivary secretory processes Saliva formed on stimulation of the sympathetic nerves or on administration of sympathetic amines is especially rich in enzymes A kinin forming enzyme kininogenase is present in the cat submandibular

salivary gland in high concentration During activation of the gland cells the kininogenase is secreted into saliva and probably also released to the interstitial fluid This enzyme has a limited proteolytical activity (Ekfors *et al* 1967) and its exocrine role is therefore disputed However within the gland the enzyme appears to play a major role in the regulation of local blood flow (Gautvik 1970 Gautvik Kriz and Lund Larsen 1972)

This study was designed to elucidate the effects of low perfusate Ca concentration on the secretion of saliva and particularly on the release of kininogenase in response to noradrenaline The incorporation of ^{14}C L-Leucine into glandular proteins under these conditions has also been measured

Materials and Methods

Surgical procedures and perfusion arrangement The experiments were carried out on the *in situ* perfused submandibular salivary gland of cats (2—4 5 kg) which had been anesthetized with i p injections of 30—40 mg/kg of Pentobarbitone (Nembutal® Abbott Laboratories London) After cannulating the trachea the arterial inflow to and the venous outflow from the gland were isolated The gland was then perfused through the lingual artery with a constant volume inflow using a peristaltic pump (Model 500—1200 M Harvard Apparatus Dover Mass) During the perfusion the carotid artery was clamped and the rate of pump perfusion adjusted so as to give the flow rate existing through the resting gland The perfusion pressure was recorded by a Statham pressure transducer (P 23 De) connected to a multitrace recorder (Grass Instrument Co Model 7 WC 12 PA Quincey Mass USA) Alterations of vascular resistance within the gland were thus reflected by changes in the perfusion pressure. The perfusion technique has been described in more detail elsewhere (Gautvik 1970) During the perfusions the effluent solution was collected and tested for the presence of kininogenase activity

Perfusates Modified Krebs-Ringer solutions were used as perfusion media Their basic composition were (in meq/l) Na 142 1 K 5 9 Mg^{2} 2 4 Cl 128 4 SO_4^{2} 2 4 HCO_3 24 7 P as phosphate 36 9 mg/l pH 7 4 Two main types of perfusates were prepared one with Ca (2 25 mmol)present and one here Ca had been replaced by isosmolar amounts of Na In special series of experiments 1 mM of ethylene glycol bis (2 aminoethyl ether) tetra acetic acid (EGTA) was added to these media

Injections into the perfusion fluid were made through a T tube connected to the poly ethylene tubing which entered the lingual artery close to the gland A calibrated syringe (Hamilton Co Whitter Cal PB 600) was used for injections of volumes of 0 05 0 1 or 0 15 ml

Intravenous injections were made through a cannula in the femoral vein *Femoral arterial blood pressure* was recorded by a Statham transducer (P 23 De) *Heparin* (Novo) 500 I U /kg was injected i v before cannulation was carried out New injections of Heparin (500 I U /kg) were carried out every econd hour

Measurements of the content of glandular kininogenase

a) *In gland homogenates* Preliminary experiments had shown that the enzyme activity varied considerably from one animal to another but that the enzyme concentration was similar in the two glands from the same animal Therefore various dilutions of a gland homogenate had to be tested and timeactivity curves had to be run for all dilutions tested The basis for the analysis was a comparison between the kininogenase activity in the two glands from the same animal For such comparisons the same degree of dilution and the same period of incubation e always used

After a perfusion experiment the salivary gland was removed and homogenized with a Dounce homogenizer for 3—5 min at 20 C in 0 1 M Tris-HCl buffer pH 8 5 (Erdo Tague and Miwa 1968) The gland homogenate was then diluted with the same buffer 50—100 times (v/v) 5 mmol (0 25 ml) of α N B nzoyl L-Arginine Ethyl Ester HCl (BAEE) we added to portions of 0 25 ml of this diluted enzyme solution After incubation at 37 15—180 min 0 1 ml of this solution was added to 0 2 ml of a freshly prepared containing equal volumes of NH OH HCl (4 M) and NaOH (? M) Then 0 1

(12 per cent (w/v) in 24 per cent HCl (w/v)) and 3 6 ml $FeCl_3$ (0 11 M) were added. The colour which developed in this solution was read at 500 nm using a Zeiss spectrophotometer

Controls without gland homogenates showed that no spontaneous hydrolysis of BAEE occurred during the maximal period of incubation The kininogenase activity was calculated as μM BAEE hydrolyzed per min and g tissue and all tests were done in duplicate

b) *In effluent perfusate* The effluent perfusates from activated and control glands were also assayed for the presence of kininogenase Usually a small contamination of venous blood was found in the outflow from the gland After centrifugation at 500 g for 10 min to remove red blood cells the perfusate was then analyzed by using the BAEE esterase method or tested directly for kinin forming activity as described below

Plasma kinin formation was used as an additional measure for evaluation of the amount of glandular kininogenase in homogenates and in effluent perfusate A kininogen II preparation purified according to Jacobsen (1966) was used as substrate for the enzyme The amount of plasma kinins liberated was measured quantitatively in terms of bradykinin equivalents on the rat uterus preparation as described elsewhere (Gautvik 1969)

Incorporation of ^{14}C L Leucine into protein fractions of glands and effluent perfusates Equal amounts of ^{14}C L-Leucine (U) (The Radiochemical Centre Amersham CFB 67 331 m Ci/mmol) were added to the perfusion media containing normal and low concentrations of Ca The glands were exposed to ^{14}C L-Leucine for a 40—60 min period and then perfused with cold perfusate for another 15 min The effluent perfusates were collected and centrifuged as described to remove red blood cells The second perfusion period with cold solution reduced the radioactivity in effluent perfusate by more than 95 per cent After the perfusion periods the glands were removed and protein fractions prepared both from the perfusate and the glands The organs were dissolved by incubation at 60 C overnight in Lowrys solution (20 g Na_2CO_3 4 g NaOH and 0 2 g Na tartrate per 1000 ml) To this solution an equal volume of TCA (10 per cent (w/v)) was added After centrifugation at 3000×g for 10 min the sediment was washed once with 5 ml of a 5 per cent (w/v) solution of TCA The sediment was then washed once with 5 ml each of absolute alcohol absolute alcohol ether 2 1 and ether respectively After evaporation the sediment was dissolved in Lowrys solution pH adjusted 7 5—8 0 and the amount of protein determined by the method of Kalckar (1947) Portions 0 5 ml where then added to 8 ml of Instagel™ (Packard) The preparation of protein fractions from effluent perfusates started with addition of TCA (10 per cent (w/v)) and did then follow the procedure described above Control additions of ^{14}C L-Leucine to glandular homogenate or perfusate followed by the above procedure for preparing protein fractions showed negligible amounts of radioactivity The radioactivity was expressed as cpm per g of protein All samples gave cpm of 5000 or more after the background activity had been subtracted

Drugs Noradrenaline (Norsk Astra A/S) was diluted with the perfusate fluid just before being used Synthetic bradykinin (BRS 640) from Sandoz was used

Statistics The levels of significance were calculated after the Student's t test two-sided

Results

The effect on saliva secretion and vascular resistance of close arterial injections of noradrenaline to the cat submandibular salivary gland

Repeated injections of noradrenaline in amounts of 0 25 to 0 50 μg in 0 05 ml (see Methods) caused secretion of saliva and vasoconstriction in glands perfused with Krebs Ringer solution containing normal amounts of Ca (Fig 1 A) The secretory responses to repeated injections of noradrenaline diminished throughout a 60 min perfusion period Towards the end of each of 4 expts the volume of secreted saliva was on the average reduced by 80 per cent while the content of protein and kininogenase diminished by 90 per cent

In glands perfused with solutions which contained no added Ca (with calculated perfusate contents of Ca of less than 0 005 mM) the secretion of saliva was reduced or abolished The vasoconstrictor effects of noradrenaline was also diminished (Fig 1 B) The saliva produced did however contain normal concentra

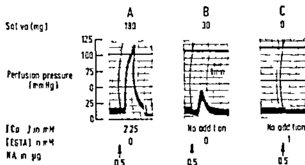


Fig 1 Secretory and vascular reactions to close arterial injections of noradrenaline (NA) in 3 submandibular salivary glands perfused at different concentrations of Ca^{++} The total amounts of saliva secreted during 12 injections of NA (total dose 6 µg) in the course of 60 min are given for each gland Typical vascular reactions to a single injection of NA are also given The glands were perfused at constant volume inflow with normal Krebs Ringer perfusate (A) with such perfusate without any added Ca^{++} (B) and with a similar perfusate containing 1 mM of EGTA and no added Ca^{++} (C) Before the injections of NA the glands had been perfused for 45 min

tions of kininogenase When EGTA was added to this latter perfusate to a final concentration of 1 mM no direct secretory or vascular responses to noradrenaline could be observed (Fig 1 C)

Effects of close arterial injections of noradrenaline on the glandular content of kininogenase It has earlier been shown that a total dose of only 1 5 µg of noradrenaline can lead to a 60 per cent reduction in the glandular content of kininogenase in submandibular gland preparations perfused with whole blood (Gautvik *et al* 1972) Hilton and Lewis (1956) were able to show that administration of sympathetic amines to this gland caused an increased kinin forming activity in the effluent perfusate Adrenergic activation of the gland is thus a powerful stimulus to the release of this enzyme from gland cells

Douglas (1968) has previously shown that low concentrations of Ca in the perfusion fluid did abolish the secretion of catecholamines from the adrenal medulla In our first series of experiments we therefore tested the effect of low Ca^{++} concentration on the noradrenaline induced release of kininogenase from the gland cells Both the submandibular glands of 5 cats were perfused with solutions to which no Ca^{++} had been added After an initial perfusion period of 30—45 min one of the glands was then activated every 5 min with injections of noradrenaline A total dose of 10—40 µg of noradrenaline was given in the course of 40—60 min As shown in Fig 2 A the activated glands showed a mean reduction in the content of kininogenase of 33 per cent ($p < 0.005$) The maximal kininogenase reduction seen in any activated gland was 64 per cent In order to achieve a further reduction in the concentration of Ca EGTA (final concentration of 1 mM) was added to the Krebs Ringer perfusion media which were already low in [Ca] This addition of EGTA should result in concentrations of free Ca^{++} near to zero while the concentrations of Mg^{++} in the perfusates were lowered to a negligible extent only concentrations of Ca and Mg^{++} were calculated using K_{Ca} $_L = 10^{7.5}$ and $10^{1.7}$ at pH 7 4 according to Ringbom (1963) As shown in Fig 2

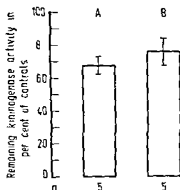


Fig 2 The effect of reductions in perfusate [Ca^{++}] on the kininogenase activity remaining in noradrenaline activated submandibular salivary glands Results from two different series of experiments are shown Total doses of nor adrenaline of 10—40 μg were given during perfusion periods of 40—60 min In each series the enzyme values from the activated glands are given as per cent of kinin ogenase activity in the perfused but non activated contra lateral control glands (means ±SE) A Glands on both sides perfused with Krebs Ringer solutions to which no Ca^{++} had been added (Enzyme activity in control glands expressed as μmol BAEE hydrolysis/g tissue×h mean 10.8×10^3 range (0.9—22) 10^3 B Glands on both sides perfused with the above solution to which EGTA (1 mM) had been added (Enzyme activity in the control glands expressed as μmol BAEE hydrolysis/g tissue×h mean 7.6×10^3 range $(0.4—12.6) \times 10^3$)

reduction in the kininogenase activity of 24 per cent ($p < 0.05$) could still be registrated in homogenates from activated glands The maximal reduction seen was 43 per cent These results show that even when [Ca] in the affluent perfusate was reduced towards zero and EGTA was present in excess the disappearance of kininogenase from activated gland cells was still not abolished

It was then attempted to find the difference in the concentration of kininogenase noradrenaline activated glands exposed to normal calcium concentration com d to activated glands exposed to conditions of low calcium The paired glands of e same animal were again used for comparisons (see Methods) The results presented in Fig 3 A show that no difference in the glandular concentration of kininogenase could be registrated between the organ which was perfused with a solution containing no added Ca and the one perfused with a normal Krebs Ringer medium

In Fig 3 B are presented the results of the same type of experiments but here EGTA (final concentration of 1 mM) had been added to both perfusion media With such an addition the normal Krebs Ringer perfusate can be calculated to contain about 1 25 mM of free calcium ions It will be seen that the concentrations of kininogenase were now considerably greater in the glands which had been perfused with solutions where [Ca] was near to zero (mean enzyme concentration 237 per cent of concentration in control glands $p < 0.05$) This means that with a grave reduction in the external Ca concentration and with in excess of free EGTA present in the perfusate the release of kininogenase from gland cells in response to stimulation by noradrenaline is markedly impaired

The glands perfused with solutions containing normal amounts of Ca^{++} secreted saliva throughout the experiments while saliva production was greatly impaired or abolished in the glands perfused with solutions of reduced Ca concentration The ability of these glands to produce saliva and also to show normal vasoconstrictor responses was however, only temporarily impaired Readdition of Ca to the perfusate or establishing normal blood circulation through the organ for a short

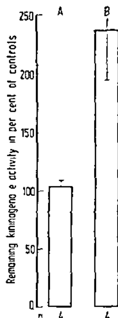


Fig 3 The effects of reductions in perfusate [Ca^{++}] on the kininogenase activity remaining in noradrenaline activated submandibular salivary glands Results from 2 different series of experiments are shown. In each series the values from the test glands are given as per cent of enzyme activity in the contralateral control glands (means ± SE) For duration of perfusion and doses of noradrenaline see Fig 2 A The glands on both sides were activated by repeated injections of NA The control glands were perfused with normal Krebs-Ringer solution The test glands were perfused with the same solution to which no Ca^{++} had been added (Enzyme activity in control glands expressed as μmol BAEE hydrolysis/g tissue × h mean 2 7 × 10^3 range (1 6—4 9) × 10^3) B The same experimental conditions as under A EGTA was however added to both perfusion media as described in Fig 2 B The control glands were thus perfused with a solution containing about 1 25 mM free Ca^{++} (Enzyme activity in control glands expressed as μmol BAEE hydrolysis/g tissue × h mean 13 8 × 10^3 range (8 0—27 2) × 10^3)

period of time restored the production of saliva and the normal vascular responses to noradrenaline injections The volume of saliva produced per stimulation was however somewhat diminished in such glands The glands often showed a small spontaneous secretion on restoring perfusate Ca or on releasing the blood circulation through the gland

The presence of kininogenase activity in effluent perfusates Plasma kinin forming activity in effluent perfusates from activated glands was assayed on the rat uterus preparation in presence of a kininase inhibitor (see Methods) Plasma kinin formation could be demonstrated up to an amount equal to 0 1 μg of bradykinin per ml No difference in the amount of kinin forming activity could be found between perfusates containing normal or low Ca concentrations

Incorporation of ^{14}C L-Leucine into glandular proteins The intracellular concentration of glandular kininogenase as of secretory proteins in general is controlled by several mechanisms *e g* synthesis catabolism and secretion Ca may be of importance at various points in these events The effect of low perfusate [Ca^{++}] on the incorporation of ^{14}C L Leucine into glandular protein was therefore determined in resting and in noradrenaline activated glands as described in Methods Fig 4 A shows the results from two groups of perfused non activated glands The control glands were perfused with the ordinary Krebs Ringer solution to which 1 mM of EGTA had been added (final concentration of Ca^{++} about 1 25 mM) The other group of glands was perfused with Krebs Ringer without added Ca^{++} but with 1 of EGTA added (final concentration of Ca near zero) It will be seen that

neurons since this phenomenon could not be observed in ganglion blocked preparations (McCarthy and Sheehan 1966) The visible production of saliva was abolished when perfusing with a medium containing negligible amounts of Ca^{++} Still the release of kininogenase as determined by measurements of enzyme activity in glandular homogenates and effluent perfusates was not blocked under these circumstances An inhibitory effect of the very low [Ca^{++}] (in the presence of EGTA) on the release of kininogenase could nevertheless be demonstrated as the glandular concentration of Krebs Ringer solution Results of this type are likely to underestimate the inhibitory this enzyme was more than twice of that in control glands perfused with normal effect of low external [Ca] for the release of this enzyme since presence of Ca is also of importance for protein synthesis *de novo* Resting glands perfused with a solution containing about 1 25 mM Ca^{++} incorporated twice as much L Leucine into glandular proteins as did glands perfused with the extremely low Ca^{++} concentration These findings show that in glands perfused with solutions containing normal or near normal amounts of Ca^{++} both the synthesis and the release of proteins are greater than in corresponding glands perfused with solutions of reduced Ca^{++} concentration Therefore the amounts of remaining kininogenase measured in the various groups of glands reflect the combined rates of secretion and synthesis of this enzyme during the experimental periods A possible variation in the rate of intracellular breakdown of the enzyme may also play a role in this respect Calcium ions are apparently of importance both for the secretion of ions (water) and of proteins from the cat submandibular gland This is in concurrence with the findings of Douglas and Poisner (1963) but the mechanisms whereby calcium exerts its effects are however still unknown The transfer of $^{45}Ca^{++}$ into or out of whole pieces of rat salivary and lacrimal glands has been studied under parasympathetic and sympathetic stimulation both *in vitro* (Dreisbach 1964) and *in vivo* (Dreisbach 1961) It was concluded from these investigations that the calcium flux in salivary glands is an active process although this has been partly contradicted by the findings of Pors Nielsen and Petersen (in press) The possible links between intraglandular calcium transport and the secretory processes have so far remained obscure The effects of calcium ions are apparently not mediated through alterations in the electrical properties of the acinus cells since normal secretory potentials can still be obtained in response to acetylcholine in the absence of calcium ions (Petersen Poulsen and Thorn 1967) The effect of external calcium on amylase secretion does not seem to be directly linked to alterations in the energy metabolism of the gland Thus deprivation of Ca^{++} blocks adrenaline induced increase in oxygen consumption of intact mouse parotid glands *in vitro* although the amylase secretion was not influenced (Hagen 1959) It also appears to be difficult to explain all the effects of external calcium a mediated through the adenyl-cyclase/cyclic AMP system Adrenaline and high potassium can thus increase cyclic AMP concentration in slices of rat parotid gland in the absence of calcium (Rasmussen and Tenenhouse 1968)

Since the hypoton saliva is mainly formed by reabsorbtion of Na and Cl in the duct lumen without an equivalent osmotic absorption of water (Martin

Young 1971) Ca might exert its action here by changing the water permeability of the cells and/or of the intercellular junctions An increase in water permeability would thus reduce the volume of secreted saliva Calcium might also be of importance for initiating the secretory response whereby ions are pumped across the gland walls The secretion of glandular kininogenase has been shown to be less sensitive to the presence of calcium ions in the perfusate than the secretion of catecholamines from the perfused suprarenal glands (Douglas 1968) Rat salivary glands contain a high concentration of calcium relative to other soft tissues (Dreisbach 1957, Kraintz 1967) Microsomal fractions from the rat parotid and submaxillary glands also showed a high calcium uptake which was not inhibited by Ouvabain (1 mM) nor 2 4 Dinitrophenol (0 5 mM) (Selinger Naim and Lasser 1970) Thus the redistribution of intracellular Ca during gland cell activation might be a key event in the secretion of enzymes and/or electrolytes in this organ in analogy to the excitation-contraction coupling occurring in muscles

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The Inhibitory Effect of Vibrations on Tension Development in Vascular Smooth Muscle

By

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In a series of papers Roach and co-workers (Roach 1963, 1970, Boughner and Roach 1971 a, b) have studied turbulence induced vibrations as a possible factor in the genesis of post stenotic dilatation. They demonstrated that vibrations altered the distensibility of isolated human arteries, a finding that was not attributed to smooth muscle activity but rather to changes in the elastin component of the vascular wall. These observations led us to examine the effects of vibrations on vascular smooth muscle tone in the freshly isolated portal vein.

The rat portal vein was isolated and mounted in an organ bath with modified Krebs solution (see Axelsson *et al.* 1967). Mechanical activity was recorded with a Grass FT 03 force transducer attached to one end of the portal vein and vibrations were induced by a modified loud speaker (vibrator) attached via a bar to the other end. A passive tension of 400 dyn was applied and the preparation was allowed to accomodate for 1 h before the experiment started. Vibrations with a maximal amplitude of 0.5 mm (corresponding to about 10—15 % of the vessel's length) were applied longitudinally to the vessel by a sine wave current of 200 Hz from a model 200 AB audio oscillator (Hewlett Packard). The amplitude of the vibrations was estimated through a dissection microscope with a micrometer eyepiece by observing the reflections from a glass indicator on the bar connecting the loud speaker with the vessel.

Responses to exogenous noradrenaline (NA) were obtained by injection of the drug (Nor-exadrin, ASTRA) into the bath and neurogenic responses were induced by transmural field stimulation (0.8 ms, 15 V).

The qualitative effects of longitudinal vibrations on spontaneous contractions and on induced electrical and NA responses were studied on six portal vein preparations. All experiments gave uniform results. Fig. 1 shows tracings from three experiments. The upper panel (A) first shows a typical spontaneous contraction and thereafter, during muscular inactivity, a small upward deflection which represents the artifact obtained by applying vibrations to this system. When, on the other hand, the vibrations were briefly applied during the two following spontaneous contractions immediate drastic reductions in tension occurred and upon cessation the contractile force was regained. The middle recording (B) shows normal spontaneous activity and an excitatory response induced by a control nerve stimulation at 8 imp/s. The same stimulation was then repeated during continuous application of vibrations. It is seen that the baseline was raised by the vibrations corresponding to the artifact and that the amplitude of each contraction was reduced to some 15 per cent of

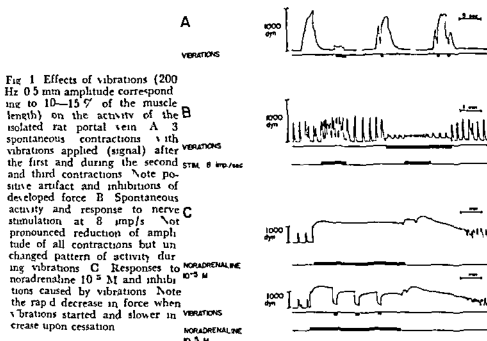


Fig 1 Effects of vibrations (200 Hz 0 5 mm amplitude corresponding to 10—15 % of the muscle length) on the activity of the isolated rat portal vein A 3 spontaneous contractions with vibrations applied (signal) after the first and during the second and third contractions Note positive artifact and inhibitions of developed force B Spontaneous activity and response to nerve stimulation at 8 imp/s Note pronounced reduction of amplitude of all contractions but unchanged pattern of activity during vibrations C Responses to noradrenaline 10^{-5} M and inhibitions caused by vibrations Note the rapid decrease in force when vibrations started and slower increase upon cessation

control level but that the pattern of activity was not altered The control activity promptly returned when the vibrations ended Fig 1 C shows the smooth contractile response of the rat portal vein to exogenous NA in a high concentration (10^{-5} M) and the abrupt decrease of the developed force as vibrations were applied (lower recording) The recovery phase had a slower time course than the inhibition In some experiments responses to high potassium ion concentration were elicited by replacing NaCl with KCl in the Krebs solution The ensuing contractures resembled the NA response (Fig 1 C) and the effects of vibrations were equally pronounced

There are apparently no previous studies on the effects of vibrations on the activity in smooth muscle However such effects on skeletal muscle have been describd (*e g* Buchthal and Kaiser 1951 p 78 Matthews 1966 Joyce Rack and Westbury 1969) and seem to be qualitatively the same as in our experiments Quantitatively the inhibition they described depends on the frequency used for stimulation of the nerves to the muscle being high at low stimulation frequencies and *vice versa* Joyce *et al* (1969) suggested that the vibratory movements increase the destruction rate of the crosslinks between thick and thin filaments within the myofibrils

The detailed organization of the contractile proteins in smooth muscle is as yet unclarified but the present results are in agreement with the explanation of Joyce *et al* (1969) since the vibrations do not affect membrane activation Thus it was seen that the pattern of phasic activity was not influenced by the vibrations (Fig 1 B), but the contractile force was reduced whether induced by a maintained d

polarization (K contracture) or associated with bursts of action potentials (phasic contractions) If the explanation of Joyce *et al* (1969) applies to the situation in smooth muscle the reason for the pronounced inhibition during vibrations found in the present experiments could be a relatively low rate of primary actomyosin formation (in agreement with the low rate of depolarisation and contraction in smooth muscle) which combined with a moderately increased destruction rate would produce a significantly decreased number of crosslinks

It should be emphasized that the mechanical artifact produced when the vibrator was switched on was of opposite direction to the inhibition, as illustrated in Fig 1 A

The present results thus demonstrate that longitudinal vibrations effectively reduce the contractile force of the isolated portal vein At present this phenomenon is being studied in some greater detail It is not yet possible to evaluate the importance of this finding for the *in vivo* situation but it seems conceivable that turbulence induced vibrations may play a role not only in the genesis of post stenotic dilatation but also in the regulation of the diameter of conduit arteries

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On the Nature of the Spindle Potential A Comment

By

D Ottoson

In his classical paper of 1950 Katz reported that the impulse response recorded from the isolated muscle spindle of the frog was superimposed upon a sustained potential which remained after blocking of the conducted activity Katz concluded that this potential represented the response of the sensory terminals of the afferent fibre (Katz 1950) This conclusion as well as later work by other authors (*cf* Ottoson and Shepherd 1965 1971) would appear to be questioned in recent papers by Ito (1970 1971 *a* *b*) In a report dealing with the effect of removal of extrafusal muscle fibres Ito concludes These results suggest that spindle potential is a composite potential of the generator potential and other unknown potentials (Ito 1970) In another paper Ito makes the following concluding statement Consequently it seems that a part of the spindle potential may consist of an artifact (Ito 1971 *a*) Similar statements may also be found in a later paper (Ito 1971 *b*) Since it is not obvious from Ito s papers that the above cited conclusions refer only to his own experiments it has been considered necessary to point this out and also to direct attention to some essential differences between Ito s experiments and those reported by Katz (1950) and ourselves (*cf* Ottoson and Shepherd 1971)

Katz emphasized in his report that it was a *sine qua non* to distinguish the receptor potential from movement artifacts and he indicated methods by which such artifacts could be identified These included short-circuiting the sensory axon (letting it lie along the muscle) or crushing it at the point of entry into the muscle (Katz 1950) In cases in which residual potentials were still recordable after this procedure Katz discarded the experiment altogether In the first report by the present author (Ottoson 1961) on the response of the isolated frog spindle the recording problems were discussed and the importance of using non polarizing electrodes was emphasized In these experiments as in all later studies the criteria used by Katz were applied (Ottoson 1961 see also Fig 1 in this paper) Thus it has been a strict rule in all our work that an experiment should be discarded if there was a residual potential in response to stretch after the nerve had been crushed The method for control routinely used may be illustrated by the records in Fig 1 Record *A* shows the receptor potential recorded from an isolated frog spindle In *B* the same stretch was applied after the sensory nerve had been crushed The absence of any resid potential changes shows that the recorded response contains no artifactual pot

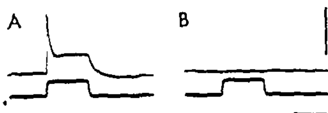


Fig 1 Response of isolated frog spindle to steplike stretch *A* before and *B* after crushing of the sensory nerve Conducted activity blocked with lignocaine (0 18 %) Vertical bar 2 mV Time bar 50 ms

It should be pointed out that in the isolated preparation movement artifacts are only rarely encountered when non polarizing electrodes are used Another factor which may be important is that the movements in the isolated preparation are relatively small compared to those in the non isolated preparation

The studies by Ito have been carried out on a comparatively gross preparation in which the spindle is not isolated and movement artifacts are therefore likely to occur In fact Ito proved that such artifacts were present in his preparation not only the magnitude but also the polarity of the response varied with the movement of the axon during stretch (Ito 1971 *a*) In the studies by Katz (1950) and in our experiments (*cf* Ottoson and Shepherd 1965) such experiments have been discarded This seems not to be the case in Itos studies His conclusion that part of the spindle potential may consist of an artifact therefore appears to be correct as far as his own recordings are concerned However this conclusion is not valid for the results reported by Katz (1950) and by the present author and his collaborators (*cf* Ottoson and Shepherd 1971)

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Reflex Inhibition of Sympathetic Nerve Activity by Phenoxybenzamine

By

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Abstract

AARS H *Reflex inhibition of sympathetic nerve activity by phenoxybenzamine* Acta physiol scand 1972 *85* 433—437

The effects on baroreceptor and sympathetic nerve activity of the α adrenergic blocking agent phenoxybenzamine have been investigated in 13 anesthetized rabbits Nerve activities were recorded at various blood pressure levels obtained by stepwise changes of blood volume With phenoxybenzamine aortic nerve activity at 80 and 90 mm Hg exceeded control values by 66 and 62 % At the same pressures renal nerve activity was reduced by 30 and 55 % Although blood pressure started to fall shortly after injecting the drug about 20 min elapsed before development of maximum effects on the nerves When studied during stepwise changes of blood pressure after a similar period of hypotension—but without the drug—sympathetic nerve activity had increased Phenoxybenzamine had accordingly effected a suppression of sympathetic activity suggesting that the hypotensive response to phenoxybenzamine is aided by increased reflex inhibition of sympathetic nerve activity

The fall in arterial blood pressure resulting from administration of α adrenergic blocking agents in considered mainly due to relaxation of vascular smooth muscle in the resistance vessels However reduction of smooth muscle tone and dilatation of vessels occurs also in other regions In the aortic arch the dilatation following injection of phenoxybenzamine leads to a substantial increase in aortic baroreceptor activity at any given blood pressure (Aars 1971 a) Ordinarily this would imply a corresponding decrease in sympathetic nerve activity suggesting that the hypotensive response to phenoxybenzamine is aided by increased reflex inhibition of the sympathetic vasomotor discharge This possibility has been investigated by recording activity in a baroreceptor nerve and a sympathetic nerve before and after injection of phenoxybenzamine into rabbits

Material and methods

Studies were performed in 13 adult rabbits anesthetized with chloralose and urethane (Aars and Akre 1968) The animals were tracheotomized and respired air Femoral or carotid artery pressure was measured with a catheter connected to a Statham transducer, and the right

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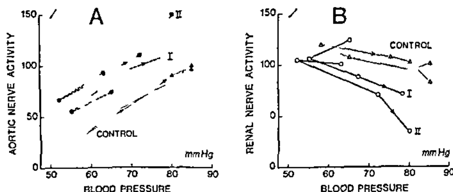


Fig 1 Relationship between aortic nerve activity and mean arterial blood pressure (A) and renal sympathetic nerve activity and blood pressure (B) before (control) and 18 (I) and 40 (II) min after injection of phenoxybenzamine (1 mg/kg) one rabbit Blood pressure changed by stepwise alterations of blood volume Arrows indicate direction of pressure change

jugular vein was used for infusions Blood pressure could be changed by alterations in blood volume A quantitative measurement of baroreceptor and sympathetic nerve activity was obtained by rectification and integration of differentially recorded activity in the intact whole left aortic and left renal nerves (Aars and Akre 1968) Neurograms integrated activity and blood pressure were recorded on a 4 channel jet ink writer (Elema Mingograph) at a paper speed of 10 mm/s Integrated activity was measured in periods of 10 s After deducting electrical noise nervous activity throughout each experiment was expressed in per cent of activity at the initial resting control blood pressure

All experiments started by determining aortic and renal nerve activity at various blood pressures in a control run The pressure was decreased and increased in steps and recordings were made after about 1 min at each level when a steady state had been reached Phenoxybenzamine (1—2 mg/kg) was then injected in 7 animals (Group A average b w 3770 g) and 6 other animals (Group B average b w 3820 g) were bled to about the same pressure levels as those following injection of phenoxybenzamine To avoid unwanted effects of severe arterial hypotension homologous heparinized blood was given to the rabbits of Group A

About 20—50 min after injecting phenoxybenzamine (Group A) or the onset of hemorrhage (Group B) nervous activities were again measured at various blood pressure levels during two experimental runs (occasionally one) separated by 10—25 min The observations were compared to control activities obtained during stepwise increases of pressure Blood pressure was raised by injecting blood or dextran if sufficient blood was not available In 3 rabbits vasopressin (0 01—0 02 units) was injected in order to obtain a blood pressure of 90 mm Hg

A quantitative study of the effects of phenoxybenzamine or hemorrhage was made by plotting the difference in nerve activity between experimental and control runs and selecting differences at mean pressures of 60 70 80 and 90 mm Hg for statistical evaluation P values were calculated based on *Student's t test*

Results

Phenoxybenzamine produced a fall in blood pressure and when examined during stepwise changes of pressure about 20 min or more after the injection increased activity in the aortic nerve and usually decreased renal nerve activity Results of a typical experiment are shown in Fig 1 Normal relationships between blood pressure and nerve activities were first established in a control run Eighteen min after injection of phenoxybenzamine blood pressure had fallen to 65 mm Hg—or 20 mm Hg below the resting control value—despite infusion of 30 ml blood Blood pressure was then altered (run I Fig 1) by removing and retransfusing 10 ml blood the

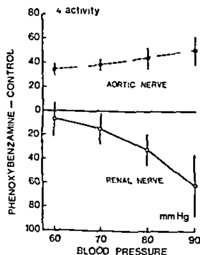


Fig 2

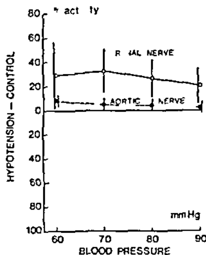


Fig 3

Fig 2 Effect of phenoxybenzamine (1—2 mg/kg) on activity in aortic and renal nerves in relation to mean arterial blood pressure Mean differences (±2 S E) between nerve activities with phenoxybenzamine and in control runs 11 expts 7 rabbits Effect of drug measured on average 28 min after injection when blood pressure was on average 26 mm Hg below mean resting control value of 95 mm Hg To prevent a larger fall in pressure 14—50 ml (mean 34) blood and dextran had been infused

Fig 3 Effect of prolonged hypotension on activity in aortic (black dots) and renal nerves (circles) Mean differences (±2 S E) between nerve activities at various blood pressures after and before hypotensive period 11 expts 6 rabbits Average duration of hypotensive periods 31 min blood pressure at this time was on average 28 mm Hg below mean resting control pressure of 94 mm Hg

highest pressure was obtained by infusing 10 ml dextran Nerve activities during the pressure increase were at this time higher than control in the aortic nerve (Fig 1 A) and lower than control in the renal nerve (Fig 1 B) Forty min after injection of phenoxybenzamine blood pressure had fallen to 63 mm Hg despite yet another infusion of 10 ml dextran Pressure was again changed (run II Fig 1) and nerve activities now *showed greater deviations from control values*

The results of 11 expts following injection of phenoxybenzamine are summarized in Fig 2 Effects of the drug are here expressed as the difference between activity with phenoxybenzamine and in the control runs Aortic nerve activity was raised at all pressure levels the mean increase at 80 and 90 mm Hg amounted to 66 and 62 % respectively of control activity Conversely renal nerve activity was decreased in all experiments at 80 and 90 mm Hg The mean reduction was significant ($p < 0.02$) at 70 80 and 90 mm Hg and was at these pressure levels 15 30 and 55 % of control activity

The effects on aortic and renal nerve activities of arterial hypotension of about the same magnitude and duration as seen after injection of phenoxybenzamine were studied in the rabbits in Group B the results of 11 expts are presented in Fig

Aortic nerve activity was generally unaffected by the hypotensive period showing a small mean increase ($p < 0.02$) at 60 mm Hg only In contrast activity in the renal nerve was increased at all pressures in most rabbits The mean rise in activity was significant ($p < 0.05$) at 70 80 and 90 mm Hg and at these pressures amounted to 33 28 and 23 % of activity during the control runs Thus without phenoxybenzamine the period of hypotension had induced changes in sympathetic nerve activity completely opposite to those seen after injection of the drug Phenoxybenzamine had therefore produced a significant ($p < 0.05$) rise in aortic baroreceptor activity and a fall in renal sympathetic nerve activity at all pressure levels examined

During all control runs at pressures between 60—90 mm Hg mean heart rate ranged from 225 to 260 beats/min with no significant differences between Groups A and B Phenoxybenzamine produced bradycardia in most animals but average changes in heart rate were small and insignificant compared with control values and changes occurring after a period of hypotension

Discussion

Phenoxybenzamine changed the relationships between blood pressure and aortic and renal nerve activities but maximum effects were not reached until nearly 20 min after the injection Long before however the peripheral action of the drug had markedly reduced blood pressure Low blood pressure—with time—is known to alter baroreceptor and sympathetic nerve activity (Beck *et al* 1966 Thamer Weidinger and Kirchner 1969 Aars 1971a Aars and Akre 1971) As the reduction in blood pressure following administration of phenoxybenzamine could be only incompletely alleviated by blood transfusion it was necessary to determine the baroreceptor and sympathetic responses to arterial hypotension of a similar degree and duration as seen after injection of the drug As shown in Fig 3 the relationship between aortic nerve activity and mean blood pressure during stepwise changes of pressure remained almost the same after the period of hypotension whereas renal nerve activity had risen 20—30 % above control values For both nerves the difference in activity at various blood pressure levels with and without phenoxybenzamine was therefore highly significant

The present results thus confirmed that phenoxybenzamine induces a rise of baroreceptor activity in the aortic nerve (Aars 1971 a) A corresponding reflex inhibition of sympathetic nerve activity would consequently be expected However integrated activity in the whole aortic nerve is predominantly a measure of activity *in the myelinated thick fibres Thinner mostly non myelinated fibres the receptors* of which are particularly sensitive to the effects of noradrenaline (Aars 1971 b) might be affected differently The possibility therefore existed that the effect on the vasomotor centres of increased discharge rate in thick fibres would be outweighed by opposite changes in the thinner fibres However this study showed that after injection of phenoxybenzamine renal sympathetic nerve activity was lower than in control runs The results therefore indicate that the net effect on the vasomotor

centres was determined by the drug induced rise of activity in myelinated baroreceptor fibres In arterial hypertension due to increased sympathetic vasomotor activity the hypotensive action of phenoxybenzamine or other α blocking agents may accordingly be assisted by increased reflex inhibition of sympathetic nerve activity

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Kininogenase Activity in the Stimulated Submandibular Salivary Gland in Cats

By

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Abstract

Gautvik K M K Nustad and J Vystyd *Kininogenase activity in the stimulated submandibular salivary gland in cats* Acta physiol scand 1972 *85* 438—445

The influence of prolonged stimulation of the chordo-lingual nerve (1 1/2—2 h) on secretion blood flow and the content of kininogenase in cat submandibular glands has been studied Quantitative estimation of hydrolysis of α N Benzoyl L-Arginine Ethyl Ester (BAEE) at pH 8 5 was found to be a useful measure of total kininogenase activity The volume of secreted saliva decreased throughout the period of chorda stimulation by as much as 30 per cent while the salivary concentration of kininogenase was reduced by up to 80 per cent The maximal increase in venous outflow as well as the duration of the chorda induced vasodilatation was also considerably reduced throughout the experiments The kininogenase activity in the glands themselves was reduced by 20—40 per cent Stimulation of the chorda caused a 5—80 per cent reduction of the kininogenase activity also in cats which had received atropine so that secretion of saliva was blocked During activation of the submandibular gland a major part of the reduction in kininogenase activity may be explained by release of enzyme into saliva However since a substantial reduction in kininogenase activity was also found in activated atropinized non secreting glands some enzyme appears to be released to the interstitial fluid whereby kinin formation may take place

Kallikrein which is probably the main kininogenase in the submandibular salivary gland in cats splits off the vasoactive peptide lysyl bradykinin (or kallidin) from specific protein precursors Soon after its discovery Ungar and Parrot (1936) proposed that this enzyme was released during glandular secretion and was the chemical mediator of the functional vasodilatation in this organ The work of Hilton and Lewis (1955 a 1955 b and 1956) on the cat submandibular salivary gland gave experimental support to the view that glandular kininogenase was involved in the vasodilatation caused by stimulation of the chorda tympani nerve These authors later proposed that kinin formation also occurred in other activated exocrine glands (Hilton and Lewis 1958 Hilton and Jones 1968) During recent years the importance of kinin formation as a mechanism involved in the functional hyperemia

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of salivary glands has been seriously questioned by several investigators These authors (Bhoola *et al* 1965 Morley Schachter and Smaje 1966 Schachter 1967 Schachter and Beilenson 1967 and 1968 Beilenson Schachter and Smaje 1968 Skinner and Webster 1968) maintain that the vasodilator effect of chorda tympani stimulation is probably mediated through the action of vasodilator nerves only

If kinin formation occurs in the stimulated submandibular gland secondary to release of kininogenase from gland cells then the concentration of this enzyme in the organ would be expected to fall during a period of gland activation even in atropinized glands The following investigation was carried out in order to test whether such a fall in organ enzyme content occurred as a result of parasympathetic nerve stimulation during atropinization Some early results from this investigation have been preliminarily presented elsewhere (Gautvik Nustad and Vystvd 1969)

Methods and materials

Surgical procedures The experiments were carried out on the perfused submandibular salivary gland of cats (weighing 2—4 kg) which had been anesthetized with i p injections of 30—40 mg/kg of pentobarbitone (Nembutal® Abbott Laboratories London) After cannulating the trachea the venous outflow from the gland was isolated and blood flow through the organ recorded by means of a photoelectric dropcounter The blood was returned to the animal via a polyethylene tubing inserted into the ipsilateral jugular vein

Intravenous injections were made through a cannula in the femoral vein

Close arterial infusions were made through a cannula in the lingual artery

Femoral arterial blood pressure was recorded with a Statham pressure transducer (P23 De)

Heparin (Novo) 500 I U /kg was injected i v before carrying out the various cannulations

Salivary secretion The submandibular ducts were exposed in the floor of the mouth and cannulated with a polyethylene tubing distal to the place where the chorda tympani nerve crosses the ducts The flow of saliva was recorded by a drop-counter The collected saliva was diluted 1 1 with a 0 9 % NaCl solution and used as a source of glandular kininogenase

Nerve stimulation The chordo-lingual nerve was dissected free cut and its distal end connected to a platinum fluid electrode The indifferent electrode was placed on one leg The nerve was stimulated supramaximally (9—14 V) with square wave pulses of 1 ms duration and at frequencies of 20 Hz The stimulations were carried out intermittently in periods of 30 s alternating with periods of 30 s without stimulation for 1 1/2—2 h

Homogenization After the period of nerve stimulation the glands on both sides were removed the non activated gland being used as controls The glands were homogenized with a Dounce hand homogenizer In some experiments the homogenate was separated into a soluble and a sedimentable fraction The homogenates were then made in 0 88 M sucrose buffered with 0 05 M Tris HCl pH 7 5 at 25 C (Erdos Tague and Miwa 1968) A 10 per cent (w/v) homogenate was prepared from the tissue The homogenate was centrifuged with a Spinco model L 2 ultracentrifuge at 9 10^6 g min at 25 C The supernatant was adjusted to a 5 per cent (w/v) solution The precipitate was washed twice and resuspended in 0 88 M sucrose giving a final 5 per cent (w/v) solution These procedures were carried out immediately after the experiment was finished The enzyme preparations were stored at −20 C overnight and assayed the following day

In other experiments the enzyme activities were determined in the whole homogenate The homogenate was then prepared in 0 25 M sucrose buffered with 0 05 M Tris HCl pH 7 5

Gel filtration was carried out on a Sephadex G 100 (AB Pharmacia, Uppsala Sweden) column (1 9 cm×50 cm) at room temperature (20—24 C) To a 5 per cent (w/v) solution of tissue homogenate was added sodium deoxycholate a d (Sigma) to a final concentration of 0 5 per cent and the solution was centrifuged at 9 10^6 g min at 4 C The supernatant was dialyzed for 6 h at 4 C before gel filtration (Visking dialysis tubing 18/32 Visking Dept Union Carbide International Co New York U S A) The supernatant was dialyzed against the same buffer which was used for equilibration of the Sephadex column (0 05 M Tris-HCl buffer of pH 8 5 with 0 1 M KCl and 0 02 per cent NaN_3 Portions of 2 ml of this dialyzed solution about 0 1 g wet weight were then applied on the Sephadex G 100 gel A flow of 10 ml/h was obtained with a LKB Peristaltic Pump (Typ Perspex 10 200)

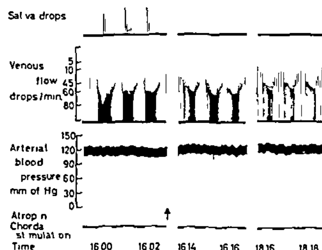


Fig 1 Effects of repeated stimulations of the chordo-lingual nerve on secretion and venous outflow from the submandibular salivary gland before and after atropine administration (↑) 0 5 mg/kg Supramaximal stimulation of the chordo lingual nerve in periods of 30 s at signals

The column was calibrated using bovine serum albumin egg albumin myoglobin and blue dextran as standards

Plasma kinin activity was determined on an isolated rat uterus as previously described (Nustad 1970)

Kininogen was prepared from citrate dog plasma as described (Nustad 1970)

Kininogenase activity Before the enzyme activity was measured in any homogenate the solution was diluted to a final concentration of from 1 20 000 up to 1 100 000 (w/v) with 0 005 M Tris-HCl buffer pH 8 5 Portions of 0 1 ml of this diluted enzyme solution were incubated with 0 1 ml of the kininogen preparation for 4 min at 37 C and 0 1 ml of this mix t re was then tested for kinin activity as described In this highly diluted homogenate no kininase activity could be detected

BAEE esterase activity was assayed as previously described (Nustad 1970)

Units of enzyme activities One unit of kininogenase activity was defined as the amount of enzyme which formed the equivalent of 1 μg of bradykinin per min under the described conditions

One unit of BAEE esterase activity corresponded to an amount of enzyme which caused the decomposition of 1 μmol of substrate per min under the standard assay conditions used

Protein amounts were determined by the biuret method (Wannemacher Banks and Wunner 1965) using bovine serum albumin (Sigma) as protein standard

Reagents

Blue Dextran 2000 (Pharmacia Uppsala Sweden)
Albumin bovine fraction V
(Sigma Chemical Company St Louis Missouri U S A)
Albumin egg grade III (Sigma)
α N Benzoyl L Arginine Ethyl Ester HCl (BAEE) (Sigma)
Triton X 100 (Octyl phenoxypolyethoxyethanol) (Sigma)
Deoxycholic acid sodium salt Sigma)
Myoglobin horse
(Nutritional Biochemical Corporation Cleveland Ohio U S A)
Bradykinin BRS 640 (Sandoz Basel Switzerland)

Results

Effects of prolonged stimulation of the chordo lingual nerve on salivary secretion and gland blood flow When the submandibular salivary glands had been activated for 1 1/2—2 h (see Methods the volume of secreted saliva was decreased by 30 per cent while the output of protein and glandular kininogenase was reduced by as

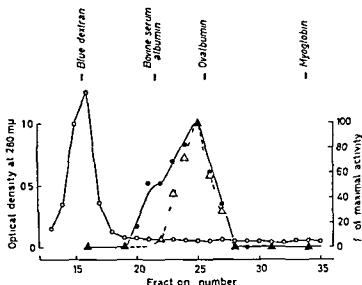


Fig 2 Kininogenase and BAEE esterase activities in homogenates from cat submandibular salivary glands Gel filtration of homogenized submandibular salivary gland carried out on Sephadex G-100 column (1 9 cm×50 cm) in 0 1 M KCl—0 05 M Tris HCl pH 8 5 as de scribed in Methods Fractions of 3 ml were collected The kininogenase (Δ——Δ) and the BAEE esterase activity (●——●) are given in per cent of maximal activity Protein (O——O) content is expressed as optical density at 280 mμ The elution volume maxima of blue dextran and reference proteins are also shown

much as 80 per cent These results are in agreement with earlier findings (Beilenson *et al* 1968) The vasodilator response to chorda stimulation was also changed during the experimental period The maximal increase in venous outflow and also the duration of the vasodilatation became reduced The same type of changes in the chorda induced vasodilatation was also observed in glands which had received atropine prior to stimulations so that secretion of saliva was blocked (Fig 1)

Relationship between the kininogenase and the BAEE esterase activity in the cat submandibular gland The concentration of the kininogenase in gland tissue was estimated by bioassaying the amount of kinin developing under standard conditions as described in Methods Kininogenases are generally also able to hydrolyze arginine esters such as α N Benzoyl L Arginine Ethyl Ester BAEE The relationship between the kininogenase activity and the BAEE esterase activity of the gland was investigated in order to see if the latter method could be used to measure the total amount of kinin forming enzymes present In 2 expts gland homogenate was gel filtered on a Sephadex G 100 column (see Methods) The kininogenase and the BAEE splitting activities were then measured in the various fractions and the maximal enzyme activities were found to concur Fig 2 However the BAEE esterase activity was somewhat more broadly distributed than that of the kininogenase activity a finding which indicates the presence of additional BAEE splitting enzymes in the gland Fig 2

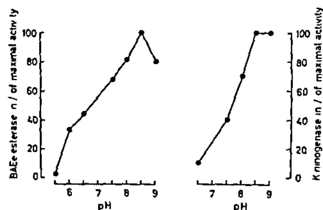


Fig 3 The effect of pH on BAEE esterase and kininogenase activity in submandibular salivary gland homogenate Enzyme activities expressed as per cent of maximal values The following buffers were used 0 15 M Tris maleate from pH 5 5 to 8 5 and 0 15 M Tris HCl at pH 9 0

The pH optimum of the kininogenase and the BAEE esterase activity were subsequently evaluated As shown in Fig 3 both the kininogenase and the BAEE splitting activity had their pH optimum at 8 5

The major part of the BAEE esterase activity measured at pH 8 5 could thus be due to that enzyme which also had the kininogenase activity In the following experiments both kininogenase activity and BAEE hydrolysis was assayed in the gland homogenates

Effects of prolonged stimulation of the chordo lingual nerve on the kininogenase and the BAEE esterase activity in gland tissue Preliminary experiments have shown that the two non activated submandibular glands from one animal have about the same weight and enzyme activity In the present tests the contra lateral un stimulated glands were therefore used as controls for the stimulated ones

In 4 cats which hade one of their submandibular salivary glands activated by chorda stimulation for 1 1/2—2 h the kininogenase activity was thus found to be reduced by 20—40 per cent Fig 4 A In 4 other cats atropine (0 5—1 mg/kg) was injected i v prior to stimulation of the chordo lingual nerve Also in these animals where the salivary secretion had been blocked one could observe a decrease in gland kininogenase activity (15—80 per cent) after nerve stimulation (Fig 4 B) Finally close arterial infusion of acetylcholine to the gland in concentrations of 4—10 μg per min for 30—45 min also caused a fall in the kininogenase activity of 30 and 50 per cent respectively (Fig 4 C) The BAEE esterase activity was reduced correspondingly in these 3 groups of activated glands as shown by Fig 5 A B and C

The kininogenase activity in the submandibular salivary gland is probably located in intracellular granules Bhoola and Ogle 1966 Bhoola 1968 Erdos *et al* 1968 Bhoola and Heap 1970) An attempt was therefore made to find out if the stimulation induced reduction in kininogenase activity was confined to the sedimental or to the soluble fraction of the gland homogenate (see Methods) Although the distribution of the kininogenase and BAEE esterase activity between these fractions varied from one animal to another stimulation of the chordo lingual nerve did as a

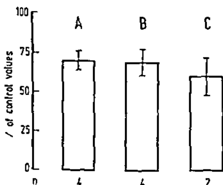


Fig 4

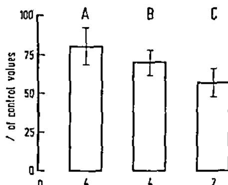


Fig 5

Fig 4 Kininogenase activity in homogenates from submandibular salivary glands tested at pH 8.5 Activity levels given in per cent of control values (M±S E) Mean control values with absolute range were A 10 0 (6 7—17 2) B 15 9 (6 8—27 2) and C 1 5 (1 4—1 6) (Units per mg protein as described in Methods) A Enzyme activity in glands activated by chorda stimulation B As group A after prior i v administration of atropine (0 5—1 mg/kg) C Enzyme activity in glands activated by close arterial infusions of acetylcholine (4—10 μg/min)

Fig 5 BAEE esterase activity in homogenized submandibular salivary glands tested at pH 8 5 Activity levels given in per cent of control values (M±S E) Mean control values with absolute range were A 0 18 (0 05—0 31) B 0 21 (0 12—0 31) and C 0 07 (0 04—0 10) (Units per mg protein as described in Methods) The groups A B and C are the same as those presented in Fig 4

rule cause a larger reduction in the enzyme content of the soluble fraction The results from measurements of the kininogenase and BAEE esterase activity were also concurrent in this series of 4 expts

Discussion

In most species including the cat submandibular gland kininogenase activity is apparently mainly due to the presence of kallikrein The enzyme which we have assayed as kininogenase is thus probably cat submandibular kallikrein although purification of the enzyme would be necessary in order to verify this assumption Our tests also show that BAEE hydrolysis at pH 8 5 appears to be an useful measure of the total kininogenase activity since kininogenase and BAEE esterase activity always changed in the same direction and to the same degree

In the active gland the intracellular concentration of kininogenase is probably determined by the speed and degree of 2 major processes synthesis and secretion The amount of kininogenase inhibitor present in the organ may also affect the concentration of active enzyme measured in the homogenate In the present experiments the glands were activated by prolonged chorda stimulation and as a consequence a reduction in the glandular kininogenase activity was found to take place Beilenson *et al* (1968) found no significant difference in the kininogenase concentration in activated salivary glands when the chordo lingual nerve was intermittently stimulated

for 25 min The discrepancy between their results and ours may be explained by the more prolonged total period of stimulation in the present tests

Large amounts of glandular kininogenase are secreted into saliva when the cat submandibular gland is activated by chorda stimulations or acetylcholine infusions A major part of the reduction in kininogenase activity during prolonged stimulation may be explained therefore as a result of enzyme release into the saliva an exocrine secretion the role of which is not as yet clarified However also in atropinized glands where saliva secretion had been blocked a substantial stimulation caused reduction in the glandular kininogenase concentration Kininogenase has been recovered in the perfusate during activation of such atropinized glands (Hilton and Lewis 1956) The reduction in enzyme activity in such glands appears therefore to be due in part to liberation of kininogenase into the interstitial space In an *in situ* preparation of the cat submandibular gland which was perfused with whole blood at constant flow rate Gautvik Hilton and Torres (1970) demonstrated that stimulation of the chorda was followed by a 20 to 60 per cent reduction of the plasma substrate for glandular kininogenase One would believe this consumption of plasma kininogen to take place in the interstitial space to where glandular kininogenase can apparently be released

Some recent experiments give further support to the idea that formation of plasma kinins takes place in the activated submandibular gland and furthermore plays a role in the chorda induced vasodilator response (Gautvik 1970 a and 1970 b Gautvik *et al* 1970) The results from these experiments (Gautvik 1970 a and 1970 b) suggested that at least two mechanisms were involved in the complete vasodilator pattern evoked by chorda stimulation The initial increase in blood flow upon such stimulation is probably caused by vasodilator nerves proper The simultaneous activation of the kinin forming system gives support to the nerve initiated vasodilatation and also maintains the increase in flow for some time after cessation of nerve stimulation (Gautvik 1970 a and 1970 b)

The results of the present experiments are in agreement with kinin formation playing such a role in the hyperaemia of the activated gland After long lasting stimulation periods both the glandular content of kininogenase and the extent of the stimulation induced vasodilatation were thus found to be reduced

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Inhibition of the Uptake of Biogenic Amines into Mast Cells by Tricyclic Psychoactive Drugs

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Abstract

FRISK HOLMBERG M *Inhibition of the uptake of biogenic amines into mast cells by tricyclic psychoactive drugs* Acta physiol scand 1972 85 446—454

The inhibitory effect of tricyclic neuroleptic and antidepressant drugs was investigated on the uptake of ^{14}C labelled 5 hydroxytryptamine (5 HT) dopamine (DA) noradrenaline (NA) and histamine (Hi) by isolated rat mast cells All drugs inhibited the amine uptake but varied quantitatively and qualitatively in their blocking effects Results showed that 5 HT and NA uptake were competitively inhibited by both neuroleptics and antidepressants whereas DA uptake only seemed competitively inhibited by neuroleptics

Tricyclic neuroleptics and antidepressants inhibit the uptake of catecholamines and 5 hydroxytryptamine *in vitro* and *in vivo* in neuronal tissue (Dengler *et al* 1961 Axelrod *et al* 1961 Iversen 1967 Carlsson 1970 and references cited Snyder 1970) and in non neuronal tissues like the thrombocytes (Marshall *et al* 1960 Stacey 1961 Todrick and Tait 1969)

It is known that rat mast cells *in vitro* take up biogenic amines *e g* 5 hydroxytryptamine (5 HT) dopamine (DA) noradrenaline (NA) and histamine (Hi) (Green 1966) Recently it was shown that chlorpromazine inhibited the uptake of these amines to mast cells (Frisk Holmberg and Uvnäs 1972)

In the present study the effects of some tricyclic psychoactive drugs (see Table I) were investigated on the uptake of 5 HT DA NA and Hi by rat mast cells *in vitro* This was done in an attempt to investigate if a relation existed between the chemical structure of these drugs and the uptake inhibition as has been reported previously for some of the drugs (Carlsson 1970

Methods

Cell isolation and calculation of ... du

Mast cells were isolated from the abdominal and pleural cavities of male Sprague Dawley rats by Ficoll density gradient centrifugation according to Uvnäs and Thon (1959) suspended and counted as previously described (Frisk Holmberg 1971)

Between 300 000 to 400 000 cells were transferred from the stock cell suspension to plastic tubes and were preincubated with or without drug for 5 min After centrifugation the supernatants were decanted and the cells were resuspended in 1 ml of incubation solution (154 mM NaCl 2 7 mM KCl 0 9 mM $CaCl_2$ and 10% (v/v) Sorensen phosphate buffer pH 7 0 ($Na_2HPO_4 + KH_2PO_4$ 67 mM) The incubation fluid had been equilibrated to incubation temperature and contained amine and drug (controls without drug) After 10 min incubation at 37 C during which the uptake is linear against time the incubation was stopped and samples handled as previously described The uptake was estimated from the amount of radioactivity found in cells after 3 washes in incubation solution as described (Frisk Holmberg and Uvnas 1972) The release of endogenous histamine was determined in each experiment The effect of drugs was investigated as far as possible on the equal amine uptake rates unless otherwise noted Therefore cells were incubated with 0 56 μM 5 HT 6 5 μM DA 19 μM NA and 99 μM Hi These amine concentrations gave approximately the same uptake rates

Determination of radioactivity was carried out as described previously (Frisk Holmberg 1971) Values are corrected for isotope dilution efficiency and background

Determination of histamine was carried out using the method of Shore *et al* (1959) with modifications as described by Frisk Holmberg (1971) None of the compounds in the concentrations used interfered with the determination of histamine

Calculations

IC_{50} (drug concentration which gave 50% inhibition of uptake) was determined on a logarithmic probability scale and ~~expressed as relative~~ activity (RA) RA

$$= \frac{1}{IC} \times 10$$

3—6 expts were performed with each drug and amine concentration Statistical significance was evaluated by t test for differences between parallel lines.

Materials

The following drugs were supplied through the courtesy of the manufacturers AB Ferrosan (Malmo Sweden) promazine HCl AB Leo (Halsingborg Sweden) chlorpromazine HCl prochlorperazine dimaleate AB Pharmacia (Uppsala Sweden) nortriptyline HCl Merck Sharpe & Dohme Inter Corp (New York USA) amitriptyline HCl protriptyline HCl Schering Corp (Bloomfield USA) fluphenazine 2 HCl perphenazine 2 HCl AB Lundbeck (Copenhagen Denmark) chlorprothixene HCl clopentixol 2 HCl flupentixol 2 HCl Geigy (Base Switzerland) imipramine HCl chlorimipramine HCl desipramine HCl Sandoz (Basle Switzerland) thioridazine HCl Pharma Rodia (Birkerod Denmark) trifluoperazine 2 HCl

Isotopes were purchased from New England Nuclear Corp (Boston USA) 5-hydroxytryptamine C^{14} binoxalate 5 HT (11 46 mCi/mmol) Radiochemical Centre (Amersham England) dopamine (ethylamine 2 C^{14}) hydrochloride DA (55 mCi/mmol) l noradrenaline (carbinol C^{14}) bitartrate NA (57 mCi/mmol) and histamine (ring 2 C^{14}) dihydrochloride Hi (54 3 mCi/mmol) Remaining chemicals were obtained from regular commercial sources

Results

All drugs gave a concentration dependent inhibition of 5 HT DA NA and Hi uptake but they differed quantitatively and qualitatively in their blocking effects (Fig 1—4) The uptake inhibition could be fully reversed if cell were washed in the incubation medium without drug

1 5 HT

The uptake of 5 HT was competitively inhibited by all the compounds studied as seen in Fig 1 a This double reciprocal plot shows 5 HT uptake with and without chlorimipramine chlorpromazine and trifluoperazine In Fig 1 b concentration response plots for some of the drugs investigated are shown As can be seen the

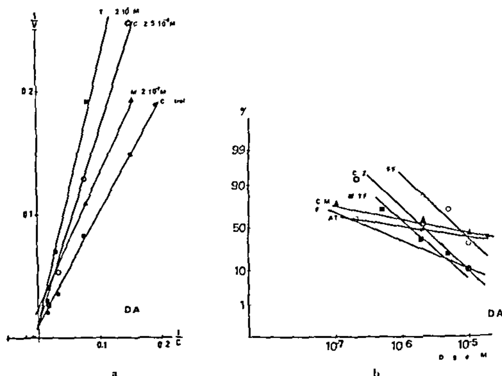


Fig 2 a Double reciprocal plots of the uptake rates (V) pmol/min/10^6 mast cells (ordinate) versus extracellular amine concentration (C) μM in a medium with or without drug Mast cells were incubated for 10 min at 37 C Regression lines were calculated according to the method of least squares Drug abbreviations[1] see page 3
Control uptake ● ——— ● DA

Fig 2 b Determination of IC_{50} values for various tricyclic psychoactive drugs Mast cells were incubated with 6 5 μM DA Symbols and text see figure legend 1 b

Significant difference was obtained between
CP—PF ($p < 0.05$ not shown in figure)
FP—FF ($p < 0.001$)
TFP—PCI ($p < 0.05$ not shown in figure)
FI—CP ($p < 0.001$ not shown in figure)
FF—PF ($p < 0.05$ not shown in figure)

contained a halogen group (Cl CF_3) in the ring system were more active than corresponding unsubstituted homologues e g chlorpromazine > promazine (see figure legend 1 b) and compounds which contained the CF_3 groups in the ring system seemed the most active of the ring halogenated derivatives

2 DA

This uptake was competitively inhibited by the neuroleptic drugs but not by any of the tricyclic antidepressants studied In Fig 2 a some double reciprocal plots and in Fig 2 b concentration response curves are shown for DA uptake

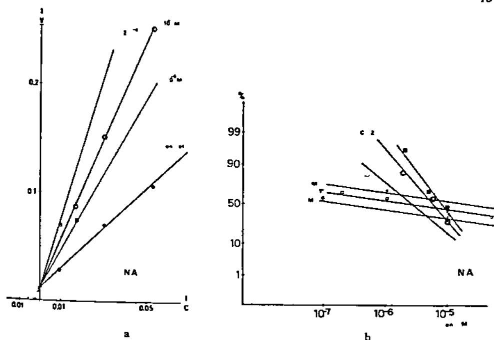


Fig 3 a Double reciprocal plots of the uptake rates (V) pmol/min/10^6 mast cells (ordinate) versus extracellular amine concentration (C) μM in a medium with or without drug Mast cells were incubated for 10 min at 37 C Regression lines were calculated according to the method of least squares Drug abbreviations[1] see page 3
Control uptake ●——● NA

Fig 3 b Determination of IC 0 values for various tricyclic psychoactive drugs Mast cells were incubated with 19 μM NA Symbols and text see figure legend 1 b Significant difference was obtained between

DMI—IMI ($p < 0\,001$)
NT—AT ($p < 0\,01$ not shown in figure)
PT—AT ($p < 0\,001$ not shown in figure)
FP—FF ($p < 0\,01$ not shown in figure)
CP—PF ($p < 0\,05$ not shown in figure)

Table I gives the IC 0 and RA values The neuroleptic drugs as a group seemed more active than the antidepressants Thioxanthenes (chlorprothixene clopentixol flupentixol) seemed more potent compounds than corresponding phenothiazine de rivatives (clopentixol > perphenazine flupentixol > fluphenazine (see figure legend 2 b)) Increasing halogenation of the tricyclic ring system (Cl to CF_3) increased the inhibitory activity (trifluoperazine > prochlorperazine fluphenazine > perphe nazine flupentixol > clopentixol (see figure legend 2 b)) Flupentixol was the most effective DA uptake inhibitor found in this study Although the antidepressant drugs seemed not to be competitive inhibitors of DA uptake some of them especially amitriptyline were effective blockers of DA uptake

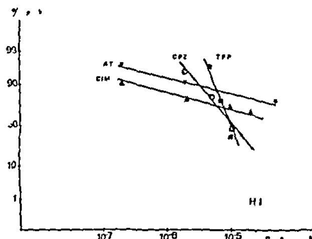


Fig 4 Effect of some tricyclic psychoactive drugs on Hi uptake (probability scale) Drug abbreviations see footnote page 3

3 NA

Both neuroleptic and antidepressant drugs inhibited NA uptake competitively (Fig 3 a) In Fig 3 b concentration response curves are shown of the same type as described before

Table I shows the IC_{50} and RA values As is seen antidepressant drugs included the most potent inhibitors of NA uptake Of these monomethylated compounds (desipramine nortriptyline protriptyline) were more active than corresponding [illegible] methylated compounds (see figure legend 3 b) Chlorimipramine was more active than imipramine Phenothiazines seemed to be weak thioxanthenes on the other hand more active inhibitors of NA uptake Neuroleptic drugs containing halogen substitution in the tricyclic ring system seemed more active than compounds without this substitution

4 Hi

The effect of some of the drugs on the uptake of histamine is seen in Fig 4 The compounds investigated reduced the uptake of Hi Phenothiazine derivatives seemed more active than antidepressant drugs In concentrations above 10^{-5} M these drugs induce histamine release from mast cells (Frisk Holmberg and van der Kleijn 1972)

Discussion

Recently it was shown that mast cells take up amines by carrier mediated energy requiring transport processes (5 HT DA and NA) and by diffusion (Hi) Chlorpromazine 10^{-7}—$10^{-[illegible]}$ M competitively inhibited the uptake of 5 HT DA and NA and also reduced the uptake of Hi (Frisk Holmberg and Uvnäs 1972)

The present results show that other tricyclic psychoactive drugs inhibit the uptake of 5 HT DA NA and Hi by mast cells

This inhibition is reversible and [illegible] both quantitatively and qualitatively for each amine The concentration response curves (Fig 1—3 b) seemed to differ in

slopes between the groups of drugs investigated (neuroleptics and antidepressants). This could indicate a different mechanism of action regarding the uptake inhibition of these drugs

5-HT and NA uptake were competitively inhibited by tricyclic neuroleptics (phenothiazines thioxanthenes) and antidepressants but the antidepressants were more active Dimethylated antidepressants were more effective 5-HT uptake blockers than monomethylated compounds which were more effective blockers of NA uptake

DA uptake was most sensitive to and competitively inhibited by neuroleptic drugs especially the thioxanthenes

The drug concentrations necessary to inhibit Hi uptake were always higher than those inhibiting the uptake of 5-HT DA and NA Tricyclic psychoactive drugs of a more lipophilic nature (Frisk Holmberg and van der Kleijn 1972 seemed more effective uptake blockers regardless of amine than less lipophilic compounds

Based on *in vitro* and *in vivo* studies it has been proposed that tricyclic antidepressant drugs cause a blockade of 5-HT and NA uptake in neuronal tissue Antidepressants which are secondary amines are supposed to be more effective NA uptake inhibitors than tertiary amines among these drugs The reverse relationship applies to 5-HT uptake (Carlsson 1970 Shaskan and Snyder 1970) From *in vivo* studies it has been concluded that antidepressants do not affect the uptake of DA (Carlsson 1970 Snyder 1970) but *in vitro* in brain synaptosomes an uptake inhibition has been found which is not competitive in nature (Horn *et al* 1971 Neuroleptic drugs on the other hand seem to be more unselective inhibitors of all amine transport processes *in vitro* (5-HT NA DA) (Horn *et al* 1971 In contrast to this finding neuroleptic drugs seem to affect dopaminergic mechanisms more selectively *in vivo* (Anden *et al* 1970)

The thrombocyte which like the mastcell, can also accumulate and store monoamines (Da Prada and Pletscher 1969 has been intensively studied with regard to 5-HT uptake (Stacey 1961) In this case the uptake inhibition after treatment with tricyclic antidepressants follows the same pattern as described here for the mast cell. That means that antidepressants of which tertiary amines are more active than corresponding secondary homologues Todrick and Tait 1969 are competitive inhibitors of 5-HT uptake (Stacey 1961 The findings from neuronal tissue and thrombocytes also obtain for the mast cell Furthermore the present results show that DA uptake *in vitro* seems preferentially inhibited by tricyclic neuroleptic drugs

It is also known that phenothiazine derivatives affect the passive transport of amines (Seeman 1966 and references cited) In the present study both neuroleptics and antidepressants reduced diffusion Hi into mast cells

These findings taken together suggest that the effects of tricyclic psychoactive drugs on cell membranes could be the result of a common interaction with the membrane In other words the effects of these drugs on neuronal tissue might not be specific

Since the drug concentrations required to inhibit amine uptake in this study are within the blood concentration range found in man Seeman and Bial 196

et al 1970), it appears that the rat mast cell would be a convenient *in vitro* model for the preliminary investigation of psychoactive drugs Such studies should give information about the pharmacodynamics and the relative clinical activity of the compounds

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Left Ventricular Receptors Activated by Severe Asphyxia and by Coronary Artery Occlusion

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Abstract

THORÉN P *Left ventricular receptors activated by severe asphyxia and coronary artery occlusion* Acta physiol scand 1972 85 455—463

The activity in unmyelinated afferents from left ventricular receptors was examined in cats during shortlasting general asphyxia and during transient occlusion of one coronary artery After 40—70 s of asphyxia the receptors increased their activity The firing initially displayed a cardiac rhythm but became continuous when the asphyxia was prolonged and intensified This increased activity was closely related to a mechanical distension of the left ventricle when the nutritional supply was compromised suggesting a mechanoreceptor rather than a chemoreceptor function of the receptors Coronary occlusion also caused an activation of the ventricular receptors after a latency of about 35 s with an initial cardiac rhythmicity which became continuous with prolonged occlusions The receptor activation appeared also in this situation to be caused by the mechanical bulging of the ischemic area of the left ventricular wall rather than by chemical factors

In a recent publication a group of left ventricular receptors signalling in non medullated vagal afferents and resembling those earlier reported by Jarisch and Zotterman (1948) was described (Öberg and Thorén 1972 b) The receptors were found to be excited by distension of the ventricular wall and were therefore classified as mechanoreceptors The endings also responded heavily to i v injections of veratrum alkaloids and were for these reasons proposed to be of major importance for inducing the so called Bezold Jarisch reflex

It has recently been proposed that a Bezold Jarisch reflex is sometimes induced when the nutritional supply to the heart becomes inadequate as during severe asphyxia (Skolasinska Sandor and Kovach 1971) Further the bradycardia and hypotension sometimes seen in patients with coronary infarction have also been ascribed to a similar reflex mechanism (Costantin 1963) It was therefore considered of interest to analyse whether the mentioned ventricular mechanoreceptors became activated during asphyxia or when one coronary artery was obstructed and thus be at least in part responsible for the reflex circulatory adjustments in such situations Preliminary data from this study has been published earlier (Öberg and Thorén 1971)

Methods

19 cats anesthetized with chloralose 30—50 mg/kg b.w. were used in the present study. The animals were placed on artificial respiration and the thorax was opened through an intercostal incision in the fifth interspace prolonged over the midline by division of the sternum. The pericardium was opened and the edges suspended to the chest wall by ligatures. Snares were placed around the ascending aorta and also around either the anterior descending coronary branch or the right coronary artery. The right cardiac nerve running from the heart to the right vagal trunk in the chest was identified after removal of the right upper lung lobe and division of the azygos vein. The vagus was then cut below the entrance of the cardiac nerve. In this way the majority of afferents from pulmonary inflation receptors were severed and the identification of cardiac receptor afferents in the vagal trunk was thus greatly facilitated (Öberg and Thorén 1972 a). The common carotid arteries, the vagal and the sympathetic nerves as well as the aortic nerves were dissected free bilaterally in the neck and separated from each other for a considerable distance. The aortic nerves were then cut as far distally as possible.

Impulse activity from left ventricular receptor afferents was recorded with conventional neurophysiological techniques from thin filaments dissected from the right cervical vagal trunk. The impulse activity was displayed on a double beam oscilloscope together with the ECG. The impulse frequency in the filament was also monitored on a Grass Polygraph recorder by means of a spike counter device. For recordings of impulse activity in ventricular receptor afferents thin filaments were cautiously dissected from the right cervical vagus and placed on the recording electrodes. The presence of the left ventricular receptor afferents in the filament was tested by analysing the response to a shortlasting occlusion of the aorta, produced by tightening the snare placed around the vessel. Filaments displaying an increased activity with this procedure were subjected to further dissection until one or a few active fibres remained. The localization of the receptors were further established by stroking the exposed epicardial surface of the heart with a plastic rod. For details see also Öberg and Thorén (1972 a).

Arterial blood pressure was measured from one femoral artery and recorded on a Grass Polygraph 7 A recorder by means of a Statham pressure transducer. In most experiments the left ventricular pressure was similarly measured from a catheter (PE 90) advanced into the ventricular cavity from one carotid artery. Heart rate was measured on the Polygraph recorder via a tachograph that was triggered by the rapid systolic upstroke of the ventricular pressure curve. In three experiments the changes in ventricular volume were followed by placing the heart in a cardiometer which was made essentially airtight by means of a rubber membrane embracing the heart smoothly around the atrioventricular grove. The cardiometer was connected to a Grass PT5 A volume transducers and recordings made on the Grass Polygraph recorder. Asphyxia was produced by switching off the respirator for variable time periods usually lasting 40—90 s. Coronary artery obstruction was produced by tightening the snare around one of the main coronary arteries for 1—2 min.

Results

Receptor responses to asphyxia

Effects of asphyxiation of the animal for 40—90 s on receptor activity was tested in 32 filaments of which 7 were single fibre preparations. 7 of the filaments did not show any altered activity whatsoever during asphyxia, while the remaining 25 filaments (5 single fibre preparations) increased their activity markedly during the asphyxia period. Fig. 1 shows recordings from a representative experiment where impulse activity in a single receptor afferent is analyzed. The record of the spike frequency indicates a very low spontaneous receptor activity and the neurogram recorded during control conditions (A) illustrates the irregular type of firing. With the onset of asphyxia there is a rise of blood pressure and of left ventricular systolic pressure but initially no change of receptor activity. With continued asphyxia the blood pressure starts to fall conspicuously and simultaneously left ventricular diastolic pressure rises considerably while the receptor activity is markedly enhanced. In phase with these events the heart rate becomes reduced. The augmented receptor discharge

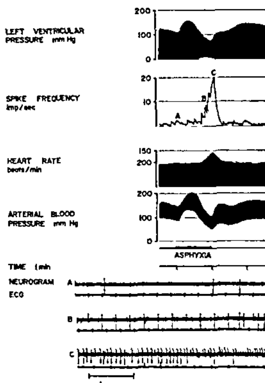


Fig 1 Effect of asphyxia (the respirator was switched off) on left intraventricular pressure arterial blood pressure heart rate and spike frequency in one unmyelinated cardiac afferent fibre (neurogram A) An increased activity is not obtained until signs of cardiac failure develop i e fall in blood pressure and elevation in left ventricular diastolic pressure The receptor first fired with cardiac rhythm (B) and then continuously (C) The increased activity is accompanied by bradycardia (The discrepancy between the arterial and left ventricular systolic pressures is due to a damping in the ventricular pressure recording system)

initially assumes a clearcut cardiac rhytm (neurogram B) but with prolonged asphyxia there is a more or less continuous and intense firing all through the cardiac cycle (neurogram C) With cession of the asphyxia period blood pressure intraventricular systolic and diastolic pressures and heart rate return to normal and receptor activity rapidly declines to control

Basically the same receptor response to asphyxia as described in Fig 1 was obtained in 23 recordings There was thus generally a considerable latency ranging from 40—90 s after the onset of asphyxia before the receptors started to fire more conspiciously This receptor excitation regularly coincided with the appearance of signs of heart failure such as a clearcut elevation of intraventricular diastolic pressure or a fall in blood pressure Initially the increased receptor discharge during asphyxia often displayed a cardiac rhythm and the nerve endings then seemed to be excited during diastole However a more precise correlation of receptor activity to a specific event in the cardiac cycle is difficult to establish because the conduction time from the receptor to the recording electrode cannot be accurately measured When receptor excitation became intense with prolonged asphyxia the discharge usually became more or less continuous The maximal discharge rate during asphyxia varied in the individual preparations between 3—20 spikes/s Only in 2 filaments (multifibre preparations) there was evidence of an increased activity early in the asphyxia period before any clearcut signs of heart failure were evident

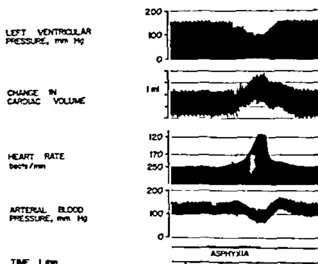


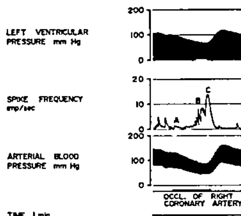
Fig 2 Effects of asphyxia on left ventricular pressure ventricular volume blood pressure and heart rate A significant change in cardiac volume elevation of left ventricular diastolic pressure and bradycardia is obtained first after 50 s of asphyxia.

From the experiment described in Fig 1 it seems as if gross alterations in the cardiac dynamics based on acute myocardial failure were required to excite the receptors Since these receptors are known to be activated by a distension or dilatation of the ventricles (Öberg and Thorén 1972 b) it was considered of interest to analyse whether the acute cardiac failure in asphyxia was accompanied by a diastolic dilatation of the heart which is highly likely since left ventricular diastolic pressure rises considerably For this purpose ventricular volume changes were recorded by means of a radiometer in 3 asphyxia experiments together with intraventricular and arterial blood pressures and heart rate Records from one of these experiments are shown in Fig 2 It is seen that after the usual latency of approximately 50 s clear signs of heart failure develop as indicated by a rapid fall in blood pressure a slowing of the heart and a rise of left ventricular diastolic pressure At the same time the diastolic and systolic ventricular volumes start to increase indicating a considerable distension of the heart Because of technical difficulties no recordings of afferent impulse activity were made in these experiments but it is reasonable to assume that also in these cases there is an increased receptor activity in connection with the elevation of the ventricular diastolic pressure (*cf* Fig 1) The pronounced bradycardia observed in the later stages of asphyxia is possibly in part a reflex effect of this increased ventricular receptor activity since the response was markedly reduced by cooling of the cervical vagi The slowing of the heart during asphyxia is therefore not only a matter of local myocardial factors

Receptor responses to occlusion of one coronary artery

Receptor activity during occlusion of one main coronary artery was tested in 8 vagal nerve filaments (4 single fibre preparations The anterior descendent coronary artery was obstructed in 4 expts and the right coronary artery in the remaining 4

Results from an experiment in this series is shown in Fig 3 Recordings were



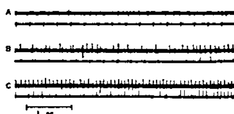


Fig 3 Effects of occlusion of the right coronary artery on left ventricular pressure blood pressure and spike frequency in two unmyelinated cardiac afferent fibres The receptors are almost silent normally (neurogram A) but are activated after about 40 s occlusion first with cardiac rhythm (B) and the continuously (C)

made from a filament containing 2 afferent fibres of which both are almost completely silent during control conditions (neurogram A) With occlusion of the right coronary artery there is a slow continuous fall of arterial blood pressure and of intraventricular systolic pressure After a latency of approximately 35 s left ventricular diastolic pressure starts to rise evidently reflecting increasing cardiac failure and coinciding with this diastolic pressure rise the recep or activity is markedly enhanced particularly in the receptor firing in the afferent fibre with the larger spike The increased discharge assumes initially a hinted cardiac rhythm (neurogram B) but the firing becomes later continuous (neurogram C) —Upon release of co onary obstruction the intraventricular and arterial pressures as well as receptor activity return to control values

In 5 of the 8 tested filaments similar responses as illstrated in Fig 3 were obtained Thus 25—40 s after the on et of coronary obstruction there was an increased receptor activity which initially displayed a cardiac rhythm but usually became continuous with prolonged coronary obstruction Two of the examined receptors were found to be activated by mechanical probing of the epicardial surface of the heart within an area supplied by the obstructed artery Concerning the three remaining filaments the exact localization of the receptors could not be established

In 2 expts there was a clearcut rise of intraventricular diastolic pressure at the later stages of coronary obstruction as illustrated in Fig 3 In the remaining coronary occlusion tests no clearcut elevation of the diastolic pressure could be detected with the present technique for recordings o intraventricular pressures

Discussion

Receptor activation to asphyxia

The present study has shown that a group of left ventricular receptors firing in unmyelinated vagal afferents and earlier analyzed in detail (Öberg and Thorén 1972 a, b) becomes activated during asphyxia and also when one main coronary artery is obstructed. The activation of the receptors under these conditions seems to be the result of a progressive distension of the ventricular wall when the nutritional supply is seriously compromised, rather than being an effect of chemical influence consequent to the disturbed metabolism. This idea is supported by the findings that the increased activity during asphyxia was closely correlated to a rise in left ventricular diastolic pressure and to the consequently augmented diastolic volume and that the receptors often fired with a clear cardiac rhythm when activated.— Admittedly with prolonged asphyxiation the rhythmicity disappeared and the receptors showed a continuous firing resembling that of a chemoreceptor. However such continuous receptor discharge occurs whenever the excitation of the endings becomes intense as *e.g.* with a shortlasting aortic occlusion (Öberg and Thorén 1972 b) in which situation no chemical factors are likely to become involved. Finally the observation that the present receptors started to fire after a considerable latency following the onset of asphyxia and coinciding with a clearcut ventricular wall distension while simultaneously *e.g.* aortic chemoreceptors started to discharge heavily within a few seconds of asphyxia (Öberg and Thorén 1972 a) also suggests that the ventricular receptors operate as mechanoreceptors rather than chemoreceptors.

The reflex circulatory adjustments caused by an excitation of the studied receptors are not known in detail. A reflex bradycardia was however regularly observed concomitantly with an increased receptor activity. Further, the present receptors signal in non medullated afferents running in the cardiac nerves (Öberg and Thorén 1972 b) and electrical stimulation of the non medullated afferent fibre group of the cardiac nerves produces reflex bradycardia, blood pressure fall and peripheral vasodilatation (Öberg and Thorén 1972 c). It is therefore reasonable to assume that the ventricular receptors presently studied excert a generalized inhibitory influence on the bulbar vasomotor centre and that they are to large extent responsible for the so called Bezold Jarisch reflex.

It has been proposed in several earlier studies that cardiac receptors may play a role in the reflex circulatory adjustments during asphyxia. Thus Benzinger *et al.* (1942) suggested that the circulatory collapse characterized by hypotension, bradycardia and fainting which is sometimes observed in hypoxic situations in man may represent a Bezold Jarisch reflex. Schaefer (1944) found a marked increase of activity in the cardiac nerves in cats subjected to asphyxia and proposed that this increased activity in cardiac receptors induced a Bezold Jarisch reflex. However to judge from records of the nerve activity in his study the increased activity seems to emanate mainly from atrial receptors. On the other hand the idea that the reflex circulatory adjustments to asphyxia may emanate from receptors of cardiac origin was refuted by Gopfert (1947) who [illegible] that the anoxia of the brain was the

most important factor in producing the blood pressure fall and bradycardia in asphyxia Similar results were obtained by Litwin and Skolasinska (1966) Anderson *et al* (1946) found that oxygen lack can sometimes precipitate a vasovagal syncope resembling that seen in post hemorrhagic fainting and characterized by a marked bradycardia a blood pressure fall and a forearm vasodilatation More recently Skolasinska *et al* (1971) suggested that the hemodynamic responses to hypoxia and particularly then the bradycardia was initiated from receptors within the heart

Although the previous studies have given somewhat conflicting results there seems to be some evidence that a vasovagal syncope like reaction or a Bezold Jarisch reflex can indeed be induced during asphyxia The present results suggest that left ventricular mechanoreceptors firing in non medullated vagal afferents may be of particular importance for these reflex responses, which will be studied in more detail in a subsequent paper

Thus concerning asphyxia the present experiments showed an increased receptor activity during asphyxia and this enhanced activity in the non medullated vagal afferents were consistently occuring when left ventricular diastolic pressure and volume started to increase as the first signs of cardiac failure

Receptor activation to occlusion of one coronary artery

Upon occlusion of coronary arterial branches which caused an increased receptor activity in 5 or 8 tested filaments there was clear evidence of an elevated left ventricular diastolic pressure in only 2 of the cases It should be realized however that such coronary obstructions will often lead to a disturbed function in only a rather restricted myocardial section which may not considerably disturb left ventricular function as a whole while a generalized asphyxia will no doubt compromise the function of the entire myocardium It is therefore reasonable to assume that also with coronary occlusion the excitation of the receptors is caused by a distension of the myocardial fibres at the site of the receptor localization It is known from studies in dogs that obstruction of a coronary branch leads to a bulging of the ischemic area within 20—90 s implying a localized distension of that area particularly during systole (Tennant and Wiggers 1935) This local distension may then act as the stimulus for mechanoreceptor nerve endings located in that particular part of the heart The observation that an increased receptor activity was not consistently found in all the coronary occlusions may therefore be due to the fact that the receptors studied were not always located in that myocardial section which was supplied by the obstructed coronary branch Such a rather localized disturbance of myocardial function may also explain why a significant rise in diastolic ventricular pressure was not regularly seen Also if the intact myocardium is distended during coronary occlusion as indicated by a rise in ventricular diastolic pressure other receptors outside the ischemic myocardium can probably be activated It should finally be noted that parallell with receptor activation in asphyxia the receptors also fired with a cardiac rhythm during coronary occlusion suggesting again that the receptor activation is due to mechanical rather than chemical factors

The problem of whether reflexes emanating from the heart may play a

the circulatory adjustments in connection with coronary artery occlusion has been discussed extensively in the literature. Levy and Frankel (1953), Wegria *et al* (1954), Struppler (1957) and Matthes (1962) found no evidence of a reflex influence from cardiac receptors in such situations.

Further, Agress *et al* (1957 a and b) found that the hypotensive shock observed after coronary occlusion was not relieved by vagotomy. However, Costantin (1963) reported an inhibition of sympathetic activity to the heart and to the skeletal muscle vessels as a result of coronary occlusion. This inhibition was evidently reflexly induced because it was abolished by vagotomy. Similar results were reported by Ascanio *et al* (1965). Kolatat *et al* (1967) described an increased activity in left ventricular receptors during coronary occlusion in dogs but these receptors were signalling in medullated fibres.

In clinical practice bradycardia and bradyarrhythmia are well known complications in the early course of coronary infarction and occur in 10—30 % of the patients treated in coronary care units (Kimball and Killip 1968, Gregory and Grace 1968). If the patients are examined within one hour after the onset of myocardial infarction, bradycardia is found in 32 % of the cases. Bradyarrhythmia is seen in 61 % of the cases with infarctions of the posterior wall and in 25 % in cases with infarction of the anterior wall (Adgey *et al* 1968). The reasons for this difference is unknown.

This bradycardia is probably due to an increased vagal activity because it is markedly diminished by atropine.

From the present results it is clear that the left ventricular receptors firing in unmyelinated vagal afferents are activated within 30 s after onset of acute coronary infarction. The question arises: What is the incidence of bradyarrhythmia at the very onset of acute myocardial ischemia? Can such a reflex mechanism from the described receptors be of importance for the early death in coronary infarction?

The reason for the *hypotensive shock* during myocardial infarction is unresolved. The decrease in cardiac output due to impaired myocardial function is of course a very important factor but can not alone explain the hypotension in all cases. Although there seems to be general agreement that systemic flow resistance is not decreased during myocardial infarction, there are no signs of any *compensatory increase of resistance in man's shocked patients* which has led to the conclusion that the normal compensatory mechanisms have for some reasons failed (*cf* Weil and Shubin (1968), Kuhn (1970)). It has also been suggested that one reason for the low cardiac output might be an *inadequate venous return* (*cf* Cohn 1970, Weil and Shubin 1970) which would indicate a gross reduction of venous tone and a consequent peripheral pooling of blood. It is probable that such venodilatation is elicited from the presently described receptors since electrical activation of unmyelinated afferents in the cardiac nerves appears to elicit a generalized reflex inhibition of sympathetic tone (Öberg and White 1970). These problems will be studied in more detail in a following series of experiments.

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the circulatory adjustments in connection with coronary artery occlusion has been discussed extensively in the literature Levy and Frankel (1953) Wegria et al (1954) Struppler (1957) and Matthes (1962) found no evidence of a reflex influence from cardiac receptors in such situations

Further Agress et al (1957 a and b) found that the hypotensive shock observed after coronary occlusion was not relieved by vagotomy However Costantin (1963) reported an inhibition of sympathetic activity to the heart and to the skeletal muscle vessels as a result of coronary occlusion This inhibition was evidently reflexly induced because it was abolished by vagotomy Similar results were reported by Ascanio et al (1965) Kolatat et al (1967) described an increased activity in left ventricular receptors during coronary occlusion in dogs but these receptors were signalling in medullated fibres

In clinical practice bradycardia and bradyarrhythmia are well known complications in the early course of coronary infarction and occur in 10—30 % of the patients treated in coronary care units (Kimball and Killip 1968 Gregory and Grace 1968) If the patients are examined within one hour after the onset of myocardial infarction bradycardia is found in 32 % of the cases Brady arrhythmia is seen in 61 % of the cases with infarctions of the posterior wall and in 25 % in cases with infarction of the anterior wall (Adgey et al 1968) The reasons for this difference is unknown

This bradycardia is probably due to an increased vagal activity because it is markedly diminished by atropine

From the present results it is clear that the left ventricular receptors firing in unmyelinated vagal afferents are activated within 30 s after onset of acute coronary infarction The question arises What is the incidence of brady arrhythmia at the very onset of acute myocardial ischemia? Can such a reflex mechanism from the described receptors be of importance for the early death in coronary infarction?

The reason for the *hypotensive shock* during myocardial infarction is unresolved. The decrease in cardiac output due to impaired myocardial function is of course a very important factor but can not alone explain the hypotension in all cases Although there seems to be general agreement that systemic flow resistance is not decreased during myocardial infarction there are no signs of any *compensatory increase of resistance* in many shocked patients which has led to the conclusion that the normal compensatory mechanisms have for some reasons failed *cf* Weil and Shubin (1968) Kuhn (1970) It has also been suggested that one reason for the low cardiac output might be an *inadequate venous return* (*cf* Cohn 1970 Weil and Shubin 1970) which would indicate a gross reduction of venous tone and a consequent peripheral pooling of blood It is probable that such venodilatation is elicited from the presently described receptors since electrical activation of unmyelinated afferents in the cardiac nerves appears to elicit a generalized reflex inhibition of sympathetic tone (Öberg and White 1970) These problems will be studied in more detail in a following serie of experiments

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that alterations of peripheral vascular tone and the consequent rise of total systemic flow resistance is the main cause of the blood pressure rise

This reflex rise of systemic flow resistance is evidently not caused by an uniform engagement of all vascular beds Flow resistances in the myocardium and skin are thus reported to remain unchanged during carotid occlusion (Bond and Green 1969) and the renal vessels have been proposed to be less involved in baroreceptor reflex mechanisms than e g skeletal muscle vessels (Opitz and Smyth 1937, Hartman Örskov and Rein 1937 McGiff and Aviado 1961 Lofving 1961) Few attempts have been made however, to analyse whether this differentiated engagement of the various vascular beds is due to a non uniform discharge rate in the respective vasomotor fibre supply or for example to differencies in the general organisation of the peripheral neuroeffectors with regard to density of innervation and/or sensitivity of the effector to neurogenic stimuli Lofving (1961) presented data which indicate that the reflexly governed discharge rate in muscle and renal vasomotor fibres differed when baroreceptor reflexes were elicited Hadjiminas and Öberg (1968) proposed the same explanation for the differentiated engagement of resistance and capacitance ve els in baroreceptor reflexes

The present series of experiments on cats were undertaken in order to elucidate whether the vasoconstrictor fibres to the various parallel coupled circuits of the systemic circulation became activated to differing degrees when baroreceptor reflexes were elicited For this purpose quantitative estimations of the average discharge rates in the constrictor fibres to skeletal muscle kidney intestine and skin during varying levels of baroreceptor activity were made by comparing the reflexly induced resistance vessel responses in the respective bed with those obtained with well defined electrical stimulations of the regional vasoconstrictor fibres

Circulatory responses to e g carotid occlusion are usually described as reflex effects and for convenience this terminology has been adopted in the present study It shold be realized, however that the responses to a reduced baroreceptor activity are not in a strict sense reflex effects but the result of a *withdrawal* of a reflex influence

Methods

Experiments were performed on 26 cats anesthetized with i v chloralose 40—50 mg/kg b w after induction with ether After tracheal cannulation the carotid arteries and vagal nerves were dissected free bilaterally in the neck The aortic nerves were identified at their junctions with the superior laryngeal nerves and loose ligatures placed around them

Arterial blood pressure was measured from one brachial artery by means of a Statham pressure transducer and heart rate by means of a tachograph triggered by the rapid systolic pressure rise —Blood pressure was maintained essentially constant throughout the experimental procedures by connecting the arterial side of the circulation via one femoral artery to a pressure reservoir

Calf muscle blood flow was measured in all 26 experiments by cannulating the popliteal vein and diverting the venous outflow through an optical drop chamber connected to a drop recorder unit like all other parameters writing on a Grass Model 5A polygraph The blood was returned to the animal via the opposite femoral vein

Renal blood flow was measured in 14 animals The abdomen was opened and the intestine removed The renal vein was dissected free and cannulated and the outflow passed through a drop chamber The blood was returned to the animal via the mesenteric or the external jugular vein.—Intestinal blood flow was recorded in 10 animals with the same type of technique on an isolated segment of the jejunum weighing around 30—40 g

Skin blood flow was measured in two animals by recording the outflow from the superficial saphenous vein of one hindlimb.

Flow resistance (PRU) in the various circuits was calculated from pressure and flow values and expressed as mm Hg/ml×min.

The regional vasoconstrictor fibres to the respective vascular beds, e.g. the lumbar sympathetic chain to the limb and the post ganglionic fibres running along the renal artery and superior mesenteric artery were carefully dissected free for a short distance and placed on electrodes for electrical stimulation. The nerves were bathed with paraffin oil at body temperature. The nerves were thus left with intact central connections. Stimuli were delivered from a Grass stimulator type S4 and the stimulation intensities chosen to give maximal responses for a given frequency. Stimulation frequencies were varied between 0.5 and 10 imp/s.

Baroreceptor reflexes were elicited in most experiments simply by occlusion of the carotid arteries. Varying levels of excitation of the vasomotor centre could be achieved by occluding first one and then both common carotid arteries before and after cutting of the aortic nerves and the vagal nerves. In 7 expts. baroreceptor activity was varied by alterations of the pressure in the isolated carotid sinuses which were perfused with arterial blood via a pump system. The blood was taken from one common carotid artery, passed through the pump and returned to both common carotid arteries which were cannulated in cephalic direction. Out-flow tubings were placed in the external carotid arteries and connected to the right external jugular vein. By adjustments of a screw clamp on this tubing, the intrasinusal pressure could be varied over a wide range. The intrasinusal pressure was recorded from a T-tube in the carotid artery-jugular vein shunt proximal to the screw clamp. A Windkessel chamber was connected to the tube system leading from the pump to the sinus regions so that pulsations could be damped at will. In these perfusion experiments the aortic and vagal nerves were regularly cut at the beginning of the experiment.

During the course of the experiment, arterial blood samples were taken at intervals for determinations of pH, PCO_2 and sometimes PO_2 (Radiometer, Copenhagen).

Experimental procedure. Skeletal muscle resistance responses were recorded in all experiments and served as a reference with which the concomitant reflex adjustments of the other bed were compared. A series of reflex responses of varying magnitude were first established either by altering the pressure in the isolated sinus preparations or by occluding one and then both carotid arteries before and after section of the aortic and vagal nerves. The regional vasoconstrictor fibres were then stimulated at different rates so that a wide range of neurogenic resistance vessel responses were obtained. In the case where an isolated sinus preparation was used, the intrasinusal pressure was maintained high during the periods of regional vasoconstrictor fibre stimulation to reduce the spontaneous activity in the constrictor fibres as much as possible. In most cases, e.g. when baroreceptor reflexes were elicited by carotid occlusions, regional stimulations were performed at normal resting blood pressure levels. In such cases some vasoconstrictor fibre activity was probably already present and its extent was in a few experiments evaluated by denervation of the vascular bed at the end of the experiment.

Results

Fig. 1 shows recording from one experiment where the vasoconstrictor fibre discharge rates to the kidney and the calf muscles were determined at varying levels of baroreceptor activity produced by alterations of the pressure in an isolated carotid sinus region. With stepwise reductions of intrasinusal pressure from 175 to approximately 22 mm Hg, there is, as expected, a gradual reflex increase in renal and skeletal muscle flow resistance and in heart rate. When intrasinusal pressure is again elevated, resistance in the two circuits returns towards control. Immediately following this sequence, the regional vasoconstrictor fibres to the two beds were stimulated with frequencies from 1 to 8 imp/s (right panel) while intrasinusal pressure was kept high to reduce, as far as possible, any prevailing constrictor fibre activity.

From the values for flow resistance obtained at the different rates of regional vasoconstrictor fibre stimulation, frequency-response curves for the respective

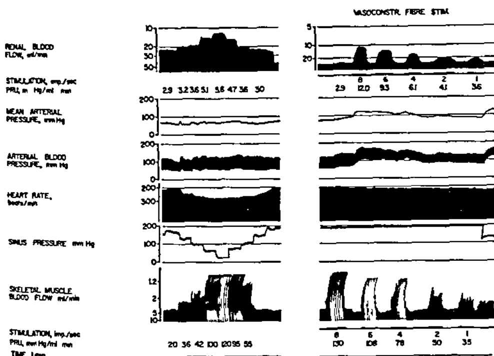


Fig 1 Effects of variations of intrasinusal pressure (left panel) and of regional vasoconstrictor fibre stimulation (right panel) on flow resistances in the kidney and the calf muscles —Blood pressure maintained essentially constant throughout The vagal and aortic nerves were cut bilaterally in the neck

vascular beds were constructed The curves obtained from the run illustrated in Fig 1 (right panel) are shown in Fig 2 In constructing the curves it was assumed that the spontaneous constrictor fibre activity was negligible at high intrasinusal pressures and that the corresponding resistance value thus implies a discharge rate of virtually 0 imp/s By plotting the flow resistance values obtained at the different intrasinusal pressure levels in the diagram an estimate of the prevailing reflexly governed constrictor fibre discharge rate could be obtained To exemplify this four values from the run shown in Fig 1 left panel i e those obtained when intrasinusal pressure was lowered to 135 (a) 100 (b) 60 (c) and 25 (d) mm Hg are plotted in the curves in Fig 2 This mode of calculation reveals that a reduction of intrasinusal pressure from 175 to 135 mm Hg causes a moderate reflex increase of vasomotor fibre discharge from zero to 1 and 0 5 imp/s in the calf muscles and the kidney respectively With reduction of the intrasinusal pressure to around 100 mm Hg the discharge rate increases to approximately 1 5 and 1 imp/s respectively in the two beds At the very lowest intrasinusal pressures there is a discharge rate of 7 imp/s in muscle vasoconstrictor fibres while at the same time the corresponding figure for the kidney is 3—4 imp/s

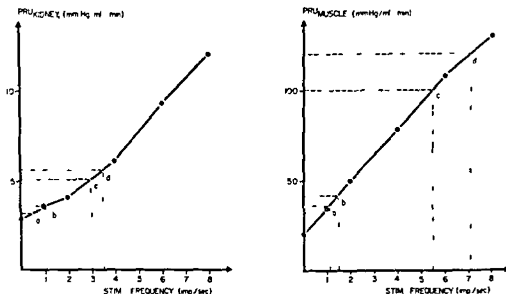


Fig 2 Frequency response curves for renal (left) and calf muscle (right) resistance vessels constructed from data obtained in the experiment shown in Fig 1 right panel Flow resistance values attained with reductions of intrasinusal pressure (Fig 1 left panel) to 135 (a) 100 (b) 60 (c) and 25 (d) mm Hg respectively are inserted in the diagrams and the reflex vasoconstrictor fibre discharge rate accordingly deduced Note the constantly lower discharge rates in the renal constrictor fibres at any given intrasinusal pressure level

The experiment illustrated in Fig 1 and 2 thus suggests that withdrawal of the baroreceptor restraint on the vasomotor centre leads to a more pronounced increase of vasoconstrictor fibre activity in skeletal muscle than in kidney It also shows that at normal sinus pressure levels *i e* around 100 mm Hg the average vasoconstrictor fibre discharge is relatively low and around 1 imp/s

Calculations and comparisons of the average constrictor fibre discharge to the calf muscles and the kidney utilizing the described approach were performed in a total of 81 baroreceptor reflex tests in 14 cats The combined results are diagrammatically illustrated in Fig 3 showing the relation between the discharge rates in muscle and renal vasoconstrictor fibres at each individual reflex test If the reflexly governed discharge rates to the two circuits were identical the values should fall along the identity line (interrupted line) It is however seen that a given impulse activity in the skeletal muscle constrictor fibres as a rule corresponds to a considerably lower discharge rate in the renal constrictor fibres In only three tests out of 81 equal discharge rates were obtained in the two sets of vasoconstrictor fibres The regression line for the data is thus found to be placed markedly to the right of the identity line A statistical analysis of the data presented in Fig 3 was performed in the following way For each individual point in the diagram the ratio between the discharge rates in muscle and kidney was calculated The mean and standard deviation of these ratios were estimated and the mean was compared in a t test

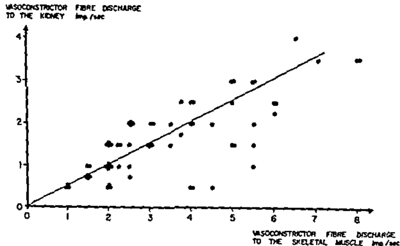


Fig 3 Relationship between the average discharge rates in calf muscle and renal vasoconstrictor fibres attained with reductions of baroreceptor activity The dots indicate individual data from 81 tests in 14 animals The heavy line is the regression line passing through the origin ($y = 0\ 4972\ x$) Broken line is the identity line ($x = y$)

with 1 (the ratio of identity) The difference between the mean ratio and 1 was then found to be highly significant ($P \ll 0\ 005$) The line passing through the origin and with a slope determined by the mean ratio represents the regression line in Fig 3

While a reduction of baroreceptor inhibitory influence on the bulbar vasomotor centre thus caused an unequal activation of the vasoconstrictor fibres to skeletal muscle and renal vessels it was found that a superimposed asphyxic excitation of the vasomotor centre led to an essentially uniformly enhanced constrictor fibre activation in the two beds (Fig 4) The animal was here asphyxiated for *2 min* by clamping the tracheal cannula which markedly increased resistance in both skeletal muscle and kidney Bilateral carotid occlusion performed shortly afterwards induces to judge from the changes in PRU a vasoconstriction of almost the same magnitude as during asphyxia in the skeletal muscles but a definitely smaller response in the kidney A comparison of alterations of PRU with those produced by regional vasoconstrictor fibre stimulation reveals that during asphyxia the vasomotor discharge rates to the two vascular beds were almost the same and exceeded 4 imp/s while with bilateral carotid occlusion alone the unequal extent of reflex activation of the two sets of vasoconstrictor fibres is again evident Thus the discharge rate to the kidney is then less than 2 imp/s while the corresponding figure for the calf muscles is in the vicinity of 4 imp/s

Such a uniform vasoconstrictor fibre activation during asphyxia as demonstrated in Fig 4 was found in all 5 expts where this maneouvre was tested As mentioned (*cf* Fig 3) also carotid occlusion alone in a few cases resulted in an equal

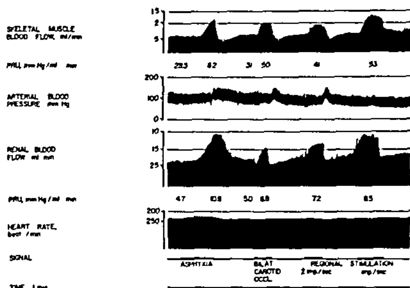


Fig 4 Effects of short lasting asphyxia bilateral carotid occlusion and regional vasoconstrictor fibre stimulation on flow resistance in skeletal muscle and the kidney Vagal and aortic nerves were cut bilaterally in the neck

of activity in muscle and renal vasoconstrictor fibres These divergent results were obtained late in the course of the experiments after a certain deterioration of the preparation was at hand and the pH of arterial blood was severely reduced some times to values below 7 1 It is conceivable that in this situation and also in the asphyxia experiments the derangement of the chemical environment of the vasomotor centre leads to an increased excitability and activity also in the central vasomotor neurons controlling the vasoconstrictor outflow to the kidneys Therefore the renal responses in such situations become augmented when the baroreceptor inhibitory influence is removed (Folkow *et al* 1961)

Impulse rate in intestinal vasoconstrictor fibres at varying levels of baroreceptor activity was estimated and compared with the concomitant discharge rate in skeletal muscle vasoconstrictor fibres in a total of 59 reflex tests in 10 cats The results from these experiments are summarized in Fig 5 which shows the relation between the estimated vasoconstrictor fibre discharge rates to the two vascular beds in each individual reflex test The values are seen to be scattered fairly evenly around the identity line and a statistical analysis similar to that performed with the kidney muscle data reveals that the mean of all individual ratios between the discharge rate in intestine and muscle was 1 0028 and that the difference between this mean ratio and 1 e g the ratio of identity was not statistically different ($P \gg 50$) The vasoconstrictor fibre activity thus increases to the same extent in skeletal muscle and intestinal vascular beds when the baroreceptor inhibitory influence on the vasomotor centre is withdrawn

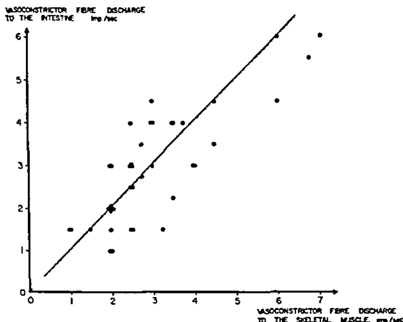


Fig 5 Relationship between the average discharge rate in calf muscle and intestinal vasoconstrictor fibres attained with reductions of baroreceptor activity The dots represent individual data from 59 tests in 10 animals The equation for the regression line (heavy line) is y = 1 003 x Broken line represents the identity line (x = y)

In 2 animals the discharge rates in the vasoconstrictor fibres to the calf muscles and to the skin of the paw were determined and compared when baroreceptor reflexes were activated Records from one experiment are shown in Fig 6 In the left panel total hind paw flow including the circulation through the arteriovenous anastomoses in the pads was measured In the right panel the pad circulation was excluded by clamping the pads with foreceps The left panel shows that with bilateral carotid occlusion there is an increased vasoconstrictor fibre activity to both the calf muscles and the paw although the average discharge rate appears to differ markedly between the two beds The reflex vasoconstriction in the muscles is thus according to the PRU values equivalent to that produced by regional vasoconstrictor fibre stimulation at a rate of approximately 2 imp/s while the reflex response in the paw vessels is considerably smaller than that obtained by regional stimulation with only 1 imp/s It should be realized however that the paw circulation is normally dominated by the arterio venous anastomoses in the pads which respond vigourously to even low frequency stimulation of the vasoconstrictor fibres (*cf* Celander 1954) The very small reflex responses of the paw vessels therefore suggests that the a v anastomoses are little or even not at all engaged in baroreceptor reflexes Instead they are in all probability reserved to participate in reflex mechanisms subserving body temperature homeostasis When the pad circulation is obstructed so that skin

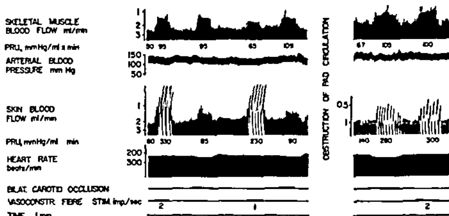


Fig 6 Effects of bilateral carotid occlusion and regional vasoconstrictor fibre stimulation on flow resistance in calf muscle and skin (hind paw) The responses before (left panel) and after (right panel) exclusion of the circulation in the a v anastomoses (obstruction of pad circulation) are shown

flow now represents flow through true nutritional vascular pathways (right panel), the reflex responses to carotid occlusion become as marked in the paw as in the skeletal muscle The vasoconstrictor fibre discharge rates in the two beds during carotid occlusion is now almost identical and according to the PRU values around 2 imp/s Similar results were obtained in repeated tests in both animals in this series The nutritional skin vessels therefore seem to become activated to the same extent as the skeletal muscle vessels when baroreceptor activity is diminished

Discussion

the present series of experiments attempts were made to estimate the discharge rate in the vasoconstrictor fibres in various parallel coupled vascular beds of the systemic circulation when their activity was reflexly augmented by unloading the arterial baroreceptors The average impulse rate was deduced by comparing the reflex constrictor responses with those obtained with direct electrical stimulation of the regional vasoconstrictor fibres with known frequencies Such an approach is evidently complicated by various disturbing factors which must be taken into account if the extent of reflex excitation of the vasoconstrictor fibres is to be estimated with any degree of exactness The reactivity of the neuroeffectors must thus be essentially identical when on one hand the reflex is elicited and on the other hand the regional stimulation is performed in order to make a comparison valid This means that the influence of *e g* vasodilator metabolites and circulating vasoactive substances and the level of transmural distending pressure must not vary to a significant extent when the two types of excitatory influences are presented These problems were hopefully surmounted in the present study by performing the reflex tests and the nerve stimulations close in time and repeatedly during the course of the experiment and at essentially identical blood pressure levels

To allow for repeated reflex tests the constrictor fibres were left with intact central connections during the experiments This means that a certain tonic spontaneous nerve activity may have been present when the electrical stimulations of the peripheral nerves were applied and that possibly a summation effect was obtained This probably is a minor problem however since the spontaneous activity seems to be normally low and in the order of 1 imp/s as shown by Lundgren *et al* (1964) Hadjiminas and Öberg (1968) and confirmed in the present study Further in those experiments where an isolated sinus preparation was used regional stimulations were generally performed during high pressure perfusion of the sinus region which will reduce or even eliminate the spontaneous vasoconstrictor fibre activity The data from such experiments did not differ significantly from the results obtained in experiments where stimulations of the vasoconstrictor fibres were performed at a sinus pressure corresponding to the normal arterial blood pressure Therefore the background activity will probably not disturb the present analysis to a significant extent

It should be noticed however that electrical stimulation of the regional vasomotor fibres implies a regional uniform activation of the whole fibre population while a reflexly induced increase of the vasoconstrictor fibre activity probably occurs as bursts of activity synchronous with blood pressure pulsations (*e g* Kehrel *et al* 1961) and possibly also with varying frequencies in different fibre units It is presently impossible to decide if and to what extent such differences in the discharge pattern in the constrictor fibres influence the vascular responses

It is furthermore possible that only a portion of the peripheral vasoconstrictor fibres were placed on the stimulation electrodes and therefore only a restricted section of the vascular bed responded to the electrical stimulations Theoretically higher stimulation rates would then be required to mimic the reflex responses where the whole fibre population is possibly activated Consequently the reflexly governed discharge rates should in this situation be overestimated However since there seems to be an extensive convergence with regard to the sympathetic innervation of circulatory effectors (*e g* Hillarp 1960) the vascular responses to electrical stimulation will probaly not be seriously affected by exclusion of a minor portion of the vasomotor fibres from the stimulation

From the considerations outlined above it is clear that the presently used approach can only give approximate information concerning the average impulse discharge rate in the vasoconstrictor fibres However for many reasons discussed at length by Lofving (1961 p 14—15) the present approach seems to be by far more feasable than application of electrophysiological techniques which theoretically should afford better possibilities for evaluations of impulse discharge rates in nerves

The present study thus demonstrates that the increased vasoconstrictor fibre discharge induced when the bulbar vasomotor centre is released from the baro receptor inhibition is not uniformly distributed to all vascular beds The renal vasoconstrictor fibres are thus activated to a considerably less extent than those supplying *e g* the skeletal muscles and the constrictor fibres to the arterio-ve

anastomoses in the pads of the paw are probably not at all engaged in baroreceptor reflexes The moderate renal response to carotid occlusion agrees with earlier findings by Lofving (1961) However in contrast to Löfvings results the present data suggest that the vasoconstrictor fibre outflow to the intestine and to the nutritional skin vessels are of the same order of magnitude as that to the skeletal muscles in baroreceptor reflexes No explanation for these partly conflicting results can presently be given

The apparently small engagement of the paw vessels in baroreceptor reflexes can as mentioned probably be explained by the fact that only a portion of the cutaneous vasoconstrictor fibres *e g* those supplying the true nutritional vessels is involved in circulatory homeostatic reflex mechanism It is possible that the slight reflex *involvement of the renal vessels can be similarly explained by an activation of only* a fraction of its vasoconstrictor fibres rather than by an over all increase of discharge rate in its entire constrictor fibre supply The kidney in comparison with skeletal muscle is a complex and heterogenous organ composed of different tissues and containing several functionally different circulatory pathways The relatively moderate changes in renal hemodynamics resulting from alterations of baroreceptor activity, might therefore conceal pronounced redistributions of the flow within the organ The possibility therefore remains that one portion of the renal vasoconstrictor bres are engaged in baroreceptor reflexes to the same extent as the vasoconstrictor ibres to the other beds while other portions of the vasomotor fibre supply to the idney are utilized by other types of reflex control mechanisms as *e g* those ncerned with regulation of blood volume However such selective activation of ly a minor portion of the renal vasomotor fibres in baroreceptor reflexes seems ss likely since under certain circumstances as *e g* when the animal is hypoventilated r acidotic for other reasons the renal vasoconstrictor responses to carotid occlusion eem to become as pronounced as those in the skeletal muscles (*cf* Folkow *et al* 961)

The background of the differentiated vasoconstrictor fibre outflow to the various parallel coupled systemic circuits when baroreceptor restraint is gradually reduced s not fully known The observations that there was a considerably lower discharge ate in renal vasoconstrictor fibres than in those running to *e g* the skeletal muscle also when the vasomotor centre was *completely* released from baroreceptor inhibitory nfluence and thus allowed to display its inherent activity suggest that the differentiation is primarily related to differencies in the level of spontaneous activity *in the separate neuron pools in the vasomotor centre The renal neuron* pools thus seem to exhibit a low inherent tonic activity under normal conditions Addition of or withdrawal of inhibitory influences will therefore normally produce only small alterations of activity in renal vasomotor fibres However during hypoventilation or acidosis the spontaneous activity in the renal vasomotor neurons evidently increases and alterations of baroreceptor activity will consequently in such situations produce more marked renal vessel responses (*cf* Folkow *et al* 1961) It is also known that a marked activation of the renal vasoconstrictor fibres also occurs when

other types of excitatory influences are placed on the vasomotor centre such as activation of non medullated pain fibres (Johansson 1962) and stimulation of the hypothalamic defence area (Feigl Johansson and Lofving 1964) The absence of baroreceptor influence on the constrictor fibres to the a v shunts in the pads can most reasonably be ascribed to the fact that these fibres pass from the hypothalamic thermoregulating centre through the brain stem without making contact with those parts of the medullary vasomotor centre which are involved in the reflex control of blood pressure

The reflex control systems serving to maintain blood pressure homeostasis are apparently so organized that they interfere little or not at all with the circulation in the kidneys and in the cutaneous a v anastomoses These vascular beds are probably rather engaged in the equally important regulation of water and electrolyte balance and of body temperature homeostasis Only in certain emergency situations such as asphyxia alarm reactions *etc* which call for extremely drastic adjustments of the circulatory system a powerful transient neurogenic interference with the renal circulation seems to occur

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Studies on Myocardial Excitation of the Right Ventricle of the Dog Heart in Situ

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Abstract

SAMUELSSON R and U SJOSTRAND *Studies on myocardial excitation of the right ventricle of the dog heart in situ* Acta physiol scand 1972 *85* 477—487

Monophasic action potentials (MAP) were recorded by a suction electrode catheter which was pushed against the endocardial side of the free wall of the right ventricle in the dog heart in situ The spatial and temporal distributions of the depolarization and repolarization processes were studied The durations of the MAP recorded from different subendocardial areas of the ventricular wall showed no systematic differences The excitation process spread radially from the prepapillary region During stimulation of the vagal nerves no change of the impulse propagation velocity or of the shape and duration of the MAP was observed On endocardial unipolar pacing in the prepapillary region of the right ventricle the excitation process in the free ventricular wall corresponded closely to that noted on spontaneous excitation this electrode position also gave the shortest Q T interval

Excellent work has been done in studies of the spread of the depolarization process in the ventricular muscle in the dog (Scher and Young 1957 Durrer Roos and Buller 1965) and in man (Durrer Roos and Buller 1965 Durrer *et al* 1970) and considerable attempts have been made to determine the temporal and spatial distributions of recovery of excitability in the ventricular muscle in the dog (Sikand *et al* 1952 Reynolds and Vander Ark 1959 Van Dam and Durrer 1961 Han and Moe 1964)

Since Wilson Macleod and Barker (1931) introduced the concept of ventricular gradient much effort has been devoted to attempts at determining the causes of the concordant polarity of the QRS complex and T wave In epicardial recordings from the canine heart the monophasic action potential obtained from the base has been found to have a longer duration than that from the apex (Schaefer Pena and Scholmerich 1943) Similarly a longer total refractory period has been reported for the basal than for the apical region in the presence of a positive T wave (Sikand *et al* 1952) A transmyocardial temperature gradient which may explain the finding of a more rapid repolarization course in the epicardial than in the endocardial muscular layer has been observed (Reynolds and Yu 1964) A shorter duration of

potentials recorded epicardially than endocardially has been found in isolated cardiac muscle preparations from the dog (Moore, Preston and Moe 1965, Edmands, Greenspan and Fisch 1966) and the frog (Lab 1971). Recording from the endocardial side of the right ventricle in man Olsson (1971) found shorter monophasic action potentials in the basal than in the apical region, which is the reverse of the findings by Schaefer *et al.* (1943) who recorded from the epicardial side of the dog heart. Relatively few *in situ* investigations have been made in which the depolarization and repolarization phases have been recorded simultaneously and where the spatial distribution in the ventricular muscle and the temporal distribution in relation to the Q-T interval of these phases have been studied (Irisawa, Ninomiya and Seyama 1966, Omura 1970, Olsson 1971).

It is known that some motor fibres of the vagal nerves are distributed within the ventricles (Truex 1970), but at present the number of such nerve fibres and their distribution are unknown (Abraham 1969, Randall 1965).

Much attention has been paid to studies of the haemodynamic conditions with different localizations of pacemaker electrodes in the ventricular muscle—epicardially in the dog (Meijler, Wieberdink and Durrer 1962, Klotz *et al.* 1963, William-Olsson and Andersen 1963, Lister *et al.* 1964, Woolfolk *et al.* 1965, Vagnini, Gourin and Stuckey 1966), subendocardially in the dog (Finney 1965) and endocardially in man (Benchimol and Liggett 1966). The impulse distribution in the ventricular muscle with epicardial pacemaker electrodes placed at different locations has been studied by epicardial recordings in the dog (Lister *et al.* 1964). To our knowledge no study of the impulse propagation in the endocardium on endocardial pacemaking has been made previously.

The aim of this paper is to present studies concerning these questions using a method described by Samuelsson and Sjöstrand (1971) — which is a modification of the suction electrode technique originally introduced by Schütz (1931)—for intracardiac recording of monophasic action potentials from the dog heart *in situ*.

Methods

The experiments were performed on 19 mongrel dogs weighing between 12 and 25 kg. After induction of anesthesia with pentobarbital (Nembutal®, 25 mg/kg) the dogs were intubated and given artificial respiration by high frequency positive pressure ventilation (Jonzon *et al.* 1971). Maintenance doses of Nembutal (on the average 50 mg/h) were administered throughout the experiments. The thorax was opened by splitting of the sternum and an approximately 10 cm long incision was made in the pericardium over the right ventricle. One or two simultaneous endocardial recordings of monophasic action potentials were made from the free wall of the right ventricle by means of the MAP catheter technique (see Fig. 1) (Samuelsson and Sjöstrand 1971). The impulses were amplified in two identical DC coupled differential amplifiers with an input impedance of 10 Mohm and recorded together with the ECG (standard lead II) on a Tektronix storage oscilloscope type 564. By using a catheter with a slightly curved tip, good contact with the endocardial surface was obtained and at the same time the risk was diminished that the external electrode would lie in direct contact with the cardiac wall (Olsson 1971). A bipolar USCI catheter (no. 5652) localized in the upper part of the right atrium was used for pacing of the heart. In the impulse propagation study with transvenous pacing of the right ventricle, a pacemaker (Elema-Schönander FMT 145) with an adjustable impulse amplitude and impulse frequency was used. The subcutaneous electrode was placed on the thoracic wall. The catheters were introduced into the heart via the femoral or jugular

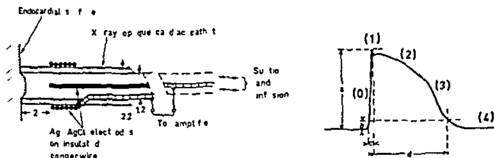


Fig 1 Left Schematic diagram showing the tip of the MAP catheter with suction against the endocardial surface Right Analysis of right ventricular MAP a = amplitude b = 10 % depolarization c = duration of phase 0 d = total duration of MAP

veins Th distal ends of the divided vagal nerves were timulated at an impulse frequency of 20 Hz (duration 2 ms amplitude 10 V) The position of the tip of the MAP catheter was established by palpation of the right ventricular wall The MAP recording was considered to be technically satisfactory when the shape of the repolarization phase was even and in agreement with microelectrode recordings presented in the literature The free wall of the right ventricle was divided into 5 areas right basal (area no 1) middle basal (area no 2) left basal (area no 3) prepapillary region (area no 4) and apex (area no 5) The amplitude (a) of an individual MAP was measured in mV and the duration in ms The rapid upstroke of the MAP was designated pha e 0 Its duration (c) was measured from the time of 10 % depolarization (b) to the initial peak of repolarizaton (phase 1) The total duration of the MAP (d) was measured at 90 % repolarization level (see Fig 1)

Results

Excitation

MAP recordings were made from 11 points (A—K) from the border areas of the free wall of the right ventricle The experiments were performed on 5 dogs (6—10 recordings from each point) The recordings were made at a high oscilloscopic sweep rate which meant that a temporal correlation between phase 0 of the MAP and certain phases of the QRS complex (40 ms) see Fig 2 was possible Recordings in which phase 0 was longer than 15 ms were discarded In the major part of the material (more than 80 % the duration of phase 0 varied between 4 and 10 ms Individual variations in the excitation course could be observed between different animals The temporal variation however of that point of phase 0 at which 10 % depolarization had taken place in relation to the QRS complex (see Fig 1) never exceeded 10 ms for any of the recording points Fig 2 shows the QRS complex (standard lead II) phase 0 and phase 1 (marked with an arrow) from representative MAP recordings for each point It is evident that the first signs of excitation were observed near the insertion of the anterior papillary muscle (G) wherefrom the wave spread over the inside of the free ventricular wall in a radial way reaching the apical part (area 5) with a lag of 15 ms and the basal part (areas 1 2 and 3) with a lag of 20—25 ms

The apical part was excited earlier in all cases than the basal parts The basal area of the conus arteriosus (area 3) was excited last

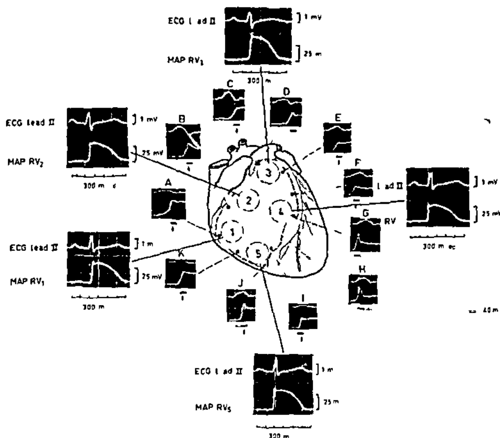


Fig 2 Endocardially recorded monophasic action potentials from the free wall of the right ventricle The larger recordings show MAPs from defined areas in relation to the ECG The smaller recordings show the depolarization phase (phase 0) and the initial part of the repolarization phase (phase 1) indicated with an arrow in relation to the duration of the QRS complex (40 ms)

Repolarization and duration of MAP

The temporal distribution of the repolarization phase in relation to the Q T interval was studied from MAP recordings from areas 1—5 in 7 dogs (9—21 recordings from each area) Furthermore in 4 dogs the mutual time relationship between the repolarization phases was studied in two simultaneous MAP recordings from areas 1 and 4 1 and 5 3 and 5 and 4 and 5 (11—20 recordings for each pair of catheters) The studies were conducted on spontaneously beating hearts Fig 2 shows representative MAP recordings in relation to the ECG (standard lead II) from areas 1—5 in the

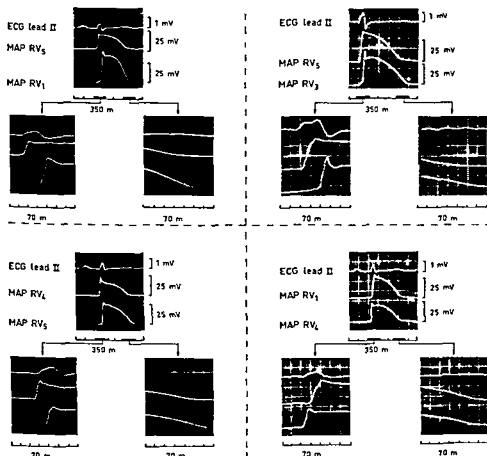


Fig 3 Simultaneous recording of ECG and two MAPs from different defined areas (see Fig 2) of the right ventricular wall In addition the depolarization pha e and the end of the repolarization phase have been recorded with a more rapid osc lloscopic sweep rate

same animal Fig 3 shows the ECG and two simultaneous MAP recordings from different areas of the right ventricular wall

The repolarization phase in the different areas began at different time points within the QRS complex and ended at different time points during the descending part of the T wave Differences in the shapes of the T waves and different degrees of negative afterpotentials in the MAP rendered difficult an exact study of the time relationship between the T wave and the end of the repolarization phase of the MAP The repolarization phase never ended before the peak of the T wave (positive or negative) and never later than the end of the T wave Area 4 depolarized and repolarized earlier than areas 1 and 5 and area 5 earlier than areas 1 and 3 (see Fig 3) This ob ervation was made both in the case of concordance and disc of polarity between the R and the T waves The MAP recordings show ed

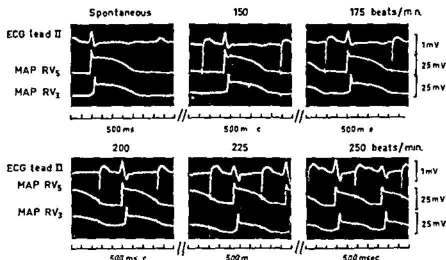


Fig 4 Simultaneous recording of ECG and two MAPs from different defined areas (see Fig 2) of the right ventricular wall Recordings from a spontaneously beating and atrially paced heart with a frequency of 150—250 beats/min are represented

narallel temporal displacement within the Q T interval The time distribution of ie depolarization phases (phase 0) within the QRS complex corresponded to that , the end of the repolarization phases in the period from the peak of the T wave ɔ its end This parallel temporal displacement between MAPs from different areas as clearly evident even at relatively high heart rates (150—250 beats/min see Fig 4)

Duration measurements of MAP recordings from areas 1—5 were made on dogs with concordance between the QRS complex and the T wave The heart was subjected to atrial pacing during the experiment with a frequency of 200 beats/min to avoid frequency dependent duration variations The results are presented in Table I No significant difference with respect to the duration of MAPs recorded from different areas ($p < 0.05$) was found in paired comparisons (Student s t test)

TABLE I Studies of the duration of MAPs recorded from 5 different defined areas (see Fig 2) of the right ventricle The recordings were made on hearts atrially paced at a constant frequency (200 beats/min) in animals with concordant polarity between the QRS complex and the T wave

Area	Number of dogs	Number of measurements	MAP duration in ms±S D
1	6	13	165±12
2	5	9	164±17
3	6	17	172±14
4	6	13	170±14
5	6	21	170±21

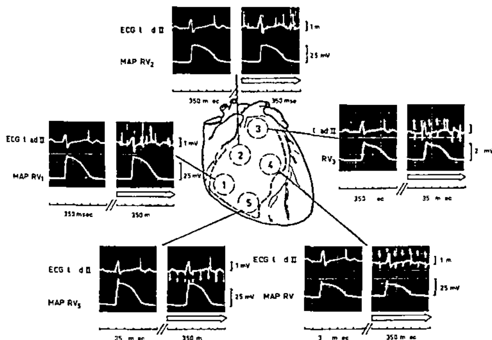


Fig 5 ECG and MAP recordings from 5 different, defined areas of the right ventricular wall before and during bilateral vagal stimulation Atrially paced heart constant frequency (200 beats/min)

Stimulation of vagus nerve

MAPs were recorded from areas 1—5 in 5 dogs before and during bilateral stimulation of the vagus nerve 8—11 recordings were made from each area and atrial pacing was performed with a frequency of 200 beats/min during the experiments to avoid frequency dependent MAP changes The recordings before and during vagal nerve stimulation were compared by superimposition some decrease in amplitude was observed in certain cases (*cf* Samuelsson and Sjostrand 1971) No change in the shape or duration of the ventricular MAP was seen during vagal stimulation (see Fig 5)

In 5 dogs 9 expts were performed in which a MAP catheter was localized to the apical area (A) and another to the conus arteriosus area (B) as illustrated in Fig 6 The heart was stimulated epicardially from the apical area of the right ventricle with the bipolar USCI catheter The time difference between phase 1 in the two MAP recordings was measured before and during bilateral stimulation of the vagus nerve In no case was there any deviation in the time difference indicating that propagation velocity and/or route of propagation were not affected by vagal stimulation

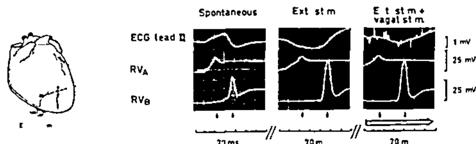


Fig 6 Simultaneous recordings of the QRS complex of the ECG and the depolarization phase (phase 0) and the initial part of the repolarization phase (phase 1) from two MAPs recorded from different defined areas of the right ventricular wall The recordings were made on the spontaneously beating heart and during epicardial ventricular stimulation (ext stim) before and during vagal stimulation Phase 1 is indicated with an arrow

Pacemaker studies

The time relationship between electrical systole (MAP) for the apical area and the outflow tract in the endocardial cell layer was studied during stimulation with the unipolar transvenous pacemaker electrode (impulse duration 2 ms impulse amplitude 1—1 5 V) localized to three different areas of the right ventricle—the apical prepapillary and conus arteriosus areas (see Fig 7) The experiments were performed on 4 dogs with 8—12 recordings for the respective electrode positions

With the pacemaker electrode placed in the prepapillary region a temporal relationship was found between MAP recordings from points A and B corresponding to that observed on spontaneous excitation When the electrode was placed in the apical area point A was excited about 30—40 ms before point B and when it was placed in the conus arteriosus area point B was excited about 30—40 ms before point A (see Fig 7) No difference in the impulse propagation velocity between points A and B on pacemaker stimulation from the apical area and from the outflow tract was noted but there was distinct prolongation of the Q T interval (the distance between the stimulation impulse and the end of the T wave) in comparison with spontaneous excitation and with pacemaker stimulation from the prepapillary region

Discussion

The spatial and temporal distributions of the excitation process in the free wall of the right ventricle as found in our investigations is in agreement with that reported by Scher and Young (1957) and Durrer Roos and Buller (1965) Our results also correspond with the finding of Durrer Roos and Buller (1965) that the excitation wave spreads radially over the inner surface of the free wall of the right ventricle starting at area trabecularis

No systematic differences in the duration of MAP recorded from different areas of the free wall of the right ventricle were found The theory that there is a systematic difference between the apical and basal areas with respect to the endocardially

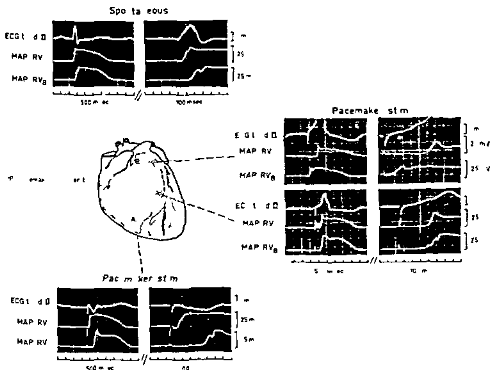


Fig 7 ECG and two MAP recordings from d fferent defined areas of the right ventricular wall The recordings were made on spontaneously beating hearts and during transvenous right ventricular stimulation from 3 d fferent areas Phases 0 and 1 were also recorded with a more rap d oscilloscopic sweep rate

recorded MAP duration (Olsson 1971) was not verified in this investigation In a few cases where two simultaneous MAP recordings were made from different areas of the cardiac wall unsystematic differences in duration were observed A possible explanation for this finding is that recordings were made from areas with different admixtures of Purkinje fibres or admixtures of damage potentials due to pressure of the outer ring electrode of the MAP catheter against the cardiac wall

In our studies with recording of endocardial MAPs phase 0 corresponds to a certain part of the QRS complex and the end of the repolarization phase correspond to a certain part of the time interval between the peak of the T wave and its end (see Fig 8 cf al o epicardial microelectrode recordings by Omura 1970) implying a parallel temporal displacement of the local MAPs in relation to the ECG

Similar to the findings of Hoffman et al (Hoffman and Suckling 1953 Hoffman and Cranefield 1960) in recordings of action potentials from the epicardial cell layer with a microelectrode technique our studies of endocardial MAP recordings show no changes of the impul e propagation velocity or of the shape or duration of the MAP during vagal stimulation in the dog heart in situ

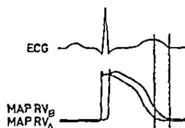


Fig 8 Diagram showing the time relationship between the ECG and endocardially recorded MAP from the right ventricular wall MAP RV$_A$ and MAP RV$_B$ represent areas that are excited early and late during the cardiac cycle

With the endocardial pacemaker electrode placed at different locations a sequency of excitation occurred which corresponded with that observed by Lister *et al* (1964) on epicardial stimulation of the right ventricle in the dog On stimulation of the prepapillary region the excitation process agreed more with that occurring spontaneously further the duration of the Q T interval was shorter than on stimulation of the apical area and of the outflow tract The shorter duration of the Q T interval on endocardial pacemaker stimulation of the prepapillary region indicates a more rapid excitation course in the ventricular muscle which may mean a more normal contraction course and more normal haemodynamics than in pacemaker stimulation with a considerably protracted Q T interval

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Relationships between Force and Velocity of Shortening in Rabbit Papillary Muscle

By

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Abstract

Edman K A P and E Nilsson *Relationships between force and velocity of shortening in rabbit papillary muscle* Acta physiol scand 1972 85 488—500

The instantaneous relationship between isotonic load and velocity of shortening was studied in rabbit papillary muscles using a quick release technique which involved critical damping of the lever The force velocity curve was determined from the experimental data by means of the least squares method using a computer program The curve exhibited a true hyperbolic shape and virtually the same values of the constants *a* and *b* in Hill s equation were obtained when the analysis was carried out during the rising or falling phase of an isometric contraction The experimental basis of the discrepant results reported in previous studies has been scrutinized Variability in active state intensity and contractile element length between individual force velocity determinations may account for the non hyperbolic shape of the force velocity curve obtained in an analysis of afterloaded contractions Quick releases performed *without* critical damping of the lever movements were found to have a depressant effect upon the myocardial activity resulting in a distortion of the force velocity curve at intermediate and small loads The distortion of the curve was more pronounced when the analysis was carried out during relaxation than during the rising phase of the contraction The deactivating effect is most likely attributable to the rapid fluctuations in speed of shortening of the contractile unit caused by inertial oscillations of the lever system after an underdamped quick release

The force velocity curve obtained for vertebrate skeletal muscle exhibits a hyperbolic shape and can be adequately described by the characteristic equation of Hill (1938) Several attempts have been made during the last few years to define the relationship between velocity of shortening and isotonic load in heart muscle (Sonnenblick 1965 a b Brady 1965 Edman and Nilsson 1968 1969 Noble Bowen and Hefner 1969 Brutsaert and Sonnenblick 1969 Parmley Yeatman and Sonnenblick 1970 Brutsaert and Sonnenblick 1971) However due to the special features of the cardiac muscle function the study of the force velocity curve has proved to be much more complicated in myocardium than in skeletal muscle and different results have been attained For discussion of the various factors that may affect the analysis of the force velocity curve in cardiac muscle see Blinks and Koch Weser (1963) Brady (1968) Edman and Nilsson (1968) Jewell and Blinks (1968) Sonnenblick and Stam (1969) and Fung (1970)

As quantitative information on the force velocity relationship is of great relevance to an understanding of the molecular events in the contractile process the problem has been taken up for further investigation To enable a meaningful evaluation of the contradictory results obtained in previous work it was considered essential to make a comparative study of the analytical techniques that are most commonly used for analysis of the force velocity curve The results have substantiated our earlier conclusion (Edman and Nilsson 1968 1969) that the force velocity relationship of cardiac muscle is a hyperbolic function Deviation from a true hyperbola would seem to be fully attributable to complications introduced by the particular techniques used Some of the results described in this paper were presented at the 1970 Gordon Conference on Heart Muscle

Methods

Muscle preparation and mounting Isolated papillary muscles of rabbits (weight < 1 2 kg) were used The techniques used for dissection of the muscles have been described (Edman and Nilsson 1968) Platinum loops were tied firmly by means of silk threads to both ends of the papillary muscle The muscle was mounted vertically in a jacketed Perspex chamber with the lower end (the ventricular wall end) attached to a force transducer (RCA 5734) which was fitted in the chamber and the upper end connected *via* a carefully straightened steel wire to an isotonic lever

The muscles used had an approximately cylindrical shape with the upper 10—15 per cent of the length (next to the insertion to the tendon) being tapered The width and length of the muscle were measured to 0 05 mm by means of an ocular micrometer at 10 × magnification while the muscle was mounted in the bath at a preload of 100 dyn In the different muscles investigated the length varied between 3 8 and 7 0 mm and the diameter between 0 5 and 1 0 mm After the muscle had been mounted in the recording chamber it was stimulated to contract isometrically at a frequency of 30 per min for at least one hour before the experiment was started A stimulation frequency of 30—48 per min was used during the actual experiment

Recording device A detailed description of the recording device including the arrangements used for stimulation of the muscle production of release and photographic recording of the signals from the transducers (1 force 2 shortening and 3 shortening velocity electrical differentiation of signal from displacement transducer) has been given previously (Edman and Nilsson 1968)

Loading of the isotonic lever was achieved in this study by means of a coil spring attached to a point close to the fulcrum of the lever The distance from the fulcrum to the point of attachment of the spring and the muscle was 2 7 mm and 54 mm respectively giving a lever ratio of 1 20 Two different levers of similar design were used during this study The equivalent mass of the levers (including the spring used for loading of the lever and the steel wire connection between lever and muscle) was 91 mg and 156 mg respectively The static friction of the levers was < 2 dyn

The stray compliance of the recording device including the connections of the muscle with the tension transducer and the lever was 0 07 m/dyn Nilsson 1972)

Damping of oscillations Oscillations of the lever were damped by means of a dash pot containing silicon oil placed on the same side of the fulcrum as the muscle A 1 mm wide and 14 mm long duralumin rod extended from the lever into the damping fluid A thin disk of aluminium (diam 3 7 mm thickness 0 4 mm) was attached perpendicular to the end of the rod The degree of damping was varied by using silicon oils of different viscosity (silicon fluid MS 200 viscosity 1000—6000 centistokes) and by altering the depth of immersion of the rod into the silicon oil With the 6000 centistokes silicon fluid it was possible to limit the velocity oscillations after quick release of the muscle to ± 0 1 lengths/sec (corresponding to approximately ± 5 per cent of the shortening velocity recorded at light load) This degree of damping as will be described below prevented the muscle from being deactivated during the release procedure It will be referred to as "critical damping" in the following and was employed in all experiments (including afterloaded contractions) unless otherwise stated

Frequency response of isotonic lever The resonant frequency of the recording system calculated from the equivalent mass and the force constant of the lever and its connections with the preparation was 350 and 290 Hz respectively for the two levers used (Nilsson 1972) These figures refer to conditions when there was no damping of the lever movement

the system was critically damped (see above) the resonant frequency of the two levers was reduced to 270 and 220 Hz respectively. After mounting of the preparation and hence introduction of extra compliance the resonant frequency of the system was further diminished. The frequency of oscillation after a damped release of an active muscle varied within the range 60—80 Hz as determined in 4 randomly chosen experiments.

Analysis of force velocity curve. The experimental data were fitted with Hill's equation by means of the least squares method using a computer program (Edman, Mulieri and Mulieri, to be published). For this purpose Hill's equation was used in the following form

$$(P+a)\ (V+b) = C$$

in which P denotes active force (dyn) and V velocity of shortening (muscle lengths/sec) and a, b and C are constants with dimensions of force, velocity and power respectively. This equation was linearized to

$$V = C\ Z - b$$

in which $Z = 1/(P+a)$. The computer program searched the appropriate values for a, b and C to minimize the mean square difference between observed and calculated velocities. Curves fitted to experimental data that were obtained by means of the "damped release technique" (quick release with critical damping of the lever system) yielded a very high correlation coefficient > 0.99.

Temperature. The temperature was kept constant to ± 0.5 C throughout an experiment by circulating water from a thermostated tank through the jacket around the muscle chamber. Between the different experiments the temperature varied within the range 28—31 C.

Solutions. A Ringer's solution of the following composition was used (mM): NaCl 120, KCl 4.0, $NaHCO_3$ 20, NaH_2PO_4 1.5, $CaCl_2$ 2.0, $MgSO_4$ 1.5, glucose 3.3, pH 7.4—7.5. The solution was continuously aerated with a mixture of 95 % O_2 and 5 % CO_2 during dissection of the muscle as well as during the actual experiment. Glass distilled water was used for washing of the glassware and for the preparation of solutions. All chemicals used were of analytical grade.

Results

The quick release technique employed in our previous work enables a detailed study of the force velocity curve in rabbit papillary muscles at a defined time during the contraction period and at a very nearly constant length of the contractile unit. An important feature of the technique needs to be emphasized at this stage, namely the fact that the lever movements are properly damped so as to minimize the inertial oscillations of the muscle length after release. As damping of the lever movements is essential in order to avoid deactivation of the muscle by the release procedure (see section II) it is appropriate to distinguish this technique from other related approaches by using the designation *damped release technique*.

I. *Force velocity curves determined from afterloaded contractions and by means of damped release technique*

The following experiments were designed to provide a quantitative comparison between force velocity curves analyzed from afterloaded contractions and from data obtained by means of the damped release technique. 6 expts. of this kind were performed, all of them giving similar results.

The damped release technique has been described in detail previously (Edman and Nilsson 1968, 1969). The system was damped to such a degree that fluctuations in shortening velocity after the release were limited to ± 0.1 lengths/sec. The frequency response of the isotonic lever was still high enough to enable adequate recording of active muscle shortening at a small load (*cf.* Methods). The principle of the analysis is illustrated in Fig. 1. The superimposed myograms show release

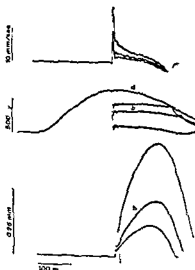


Fig 1

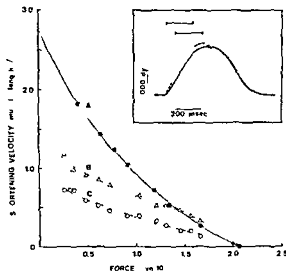


Fig 2

Fig 1 Examples of damped releases and the method used for analysis of force velocity curve. Photographically superimposed oscilloscope records of quick releases initiated immediately before attainment of peak isometric tension. Traces in order from above: Velocity of shortening, tension and shortening. Corresponding traces are denoted with the same letters a—c. Unreleased isometric myogram d. Dashed vertical line indicates time at which the data were collected, which occurs 15 ms after the release and coincides with the time of isometric peak twitch tension. Temperature 27 °C. Contraction frequency 48 per min. Resting length 3.8 mm.

Fig 2 Force velocity curves of rabbit papillary muscle obtained by means of damped release method (A) and from afterloaded contractions (B and C). Data used for construction of curve B refer to peak velocity of shortening at each respective load. Curve C is based on measurements made 10 ms after the transition from isometric to isotonic contraction. Curves B and C fitted by eye. Curve A has been drawn according to Hill's equation using a value of constant $a = 1.87 \cdot 10^3$ dyn and of $b = 2.55$ lengths/sec. The correlation coefficient between experimental data and values predicated from curve A is 0.999. Temperature 30 °C. Contraction frequency 30 per min. Resting length 6.2 mm. Insert figure indicates the isometric twitch (solid line) and the approximate time course of the active state (dashed line) as estimated from previous studies (Edman and Nilsson 1968, 1969, Nilsson 1972). Horizontal bars on top of myogram indicate time intervals for collection of the experimental data included in curve B and curve C respectively. Data included in curve A were all obtained at the time of isometric peak twitch tension.

of the muscle carried out immediately before the peak of the isometric twitch to allow the muscle to shorten against various loads. The velocity of shortening (and the corresponding tension) was measured at an instant (12—18 ms after the release in the different experiments) which coincided with the time of the isometric peak twitch tension (dotted line). While the values obtained in this way all refer to the same instant during the contraction they do represent somewhat different lengths of the contractile unit. The difference in contractile element length between the individual data used for construction of the force velocity curve did not, however, exceed 1.9 per cent of the total muscle length (for details of the calculations see Nilsson 1972).

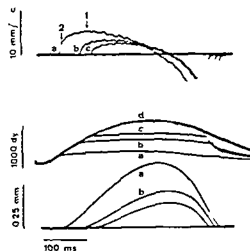


Fig. 3. Illustration of methods used for collection of force-velocity data from afterloaded contractions. Superimposed oscilloscope recordings of contractions carried out with different afterloads. Traces in order from above: Velocity of shortening, tension and shortening. Corresponding traces are denoted with the same letters a—c. Ordinary isometric myogram: d. Two different methods were used for collection of analytical data indicated by arrows on top of velocity record *a*. 1. The *maximal* speed of shortening in each record was used. 2. The speed of shortening was measured at a given time (5—10 ms) after the onset of external shortening. Temperature 28° C. Contraction frequency 48 per min. Resting length 5.5 mm.

A force-velocity curve derived from damped releases is illustrated in Fig. 2 (Curve A). The experimental values have been fitted with Hill's equation by means of a computer program (see Methods). The correlation coefficient between experimental values and points calculated from the hyperbolic curve has a high value (0.999) indicating that Hill's equation provides a very good fit. A similarly good correlation (> 0.99) is regularly obtained if the release movements of the lever are damped as stated above. The force-velocity curve (A) illustrated in Fig. 2 gives a value of V_{max} = 2.77 lengths/sec and of a/P_0 = 0.9[1].

Fig. 3 illustrates a series of recordings from afterloaded contractions of a papillary muscle. In collecting the analytical data from such records two different methods were used (see arrows above velocity record *a* in Fig. 3): 1. the maximal speed of shortening at the respective load was used; 2. the velocity of shortening was measured very early (5—10 ms) after the onset of the isotonic phase. Force-velocity curves derived from such measurements are illustrated in Fig. 2 (dashed lines), curve B being obtained according to method 1 and curve C according to method 2. The two curves refer to the same muscle as used for determination of curve A. The shape of the force-velocity curves derived from afterloaded contractions is similar to that described in several previous studies in which the same afterload recording techniques have been utilized (Brutsaert and Sonnenblick 1969, Parmley, Yeatman and Sonnenblick 1970, Brutsaert and Sonnenblick 1971). It can be seen that the curves have a sigmoid form being concave upwards at low and intermediate loads and exhibiting an upwards convexity in the region of the highest loads.

It is evident by comparing curves B and C with curve A that the afterload recording technique provides a very different picture of the force-velocity relationship

[1] It should be pointed out that the value of *a* (and *b*) remains virtually constant while P_0 changes both with the time during contraction and in response to inotropic interventions (see section II and Edman and Nilsson 1964). The ratio a/P_0 thus varies over a considerable range in cardiac muscle depending upon the value of P_0 at the moment of analysis of the force-velocity curve.

than displayed by the damped release method The main reason for the discrepant shape of the curves lies in the fact that the experimental data used for construction of curves B and C were collected at different times during the contraction period (indicated above inverted isometric myogram in Fig 2) and hence refer to different states of activity of the contractile system This is in contrast to the situation in curve A in which all data have been collected at the time of the isometric peak twitch tension The fact that the right most values of curve B fall above curve A is thus explainable on the basis that they relate to the interval preceding the attainment of isometric peak twitch tension These data therefore represent a greater intensity of the active state than the corresponding values in curve A The approximate time course of the myocardial active state (as deduced from our previous work (Edman and Nilsson 1968 1969 Nilsson 1972) is illustrated by the dashed line superimposed on the isometric myogram in Fig 2 (insert)) On similar ground the depression of the left hand portion of curve B can be accounted for by the fact that the velocity values at small loads are collected during the rising phase of the active state

The importance of a careful control of the time for collection of the analytical data is further illustrated by the difference between curve B (*maximum* shortening velocities in afterload records) and curve C (*initial* shortening velocities in afterload records) That lower velocity values are exhibited in curve C is very probably due to the fact that these data refer to an earlier time during the rising phase of the active state than the data included at each respective load in curve B

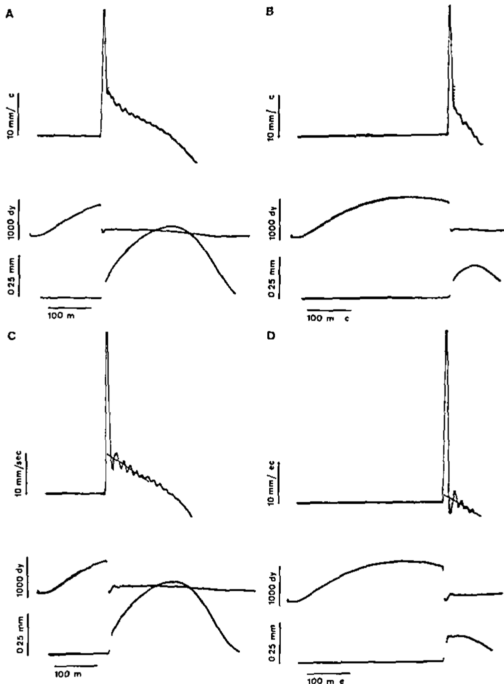
Inconstancy in contractile element length between the individual values in a force velocity curve derived from afterloaded contractions further complicates the evaluation of the curve (*cf* discussion on p 212 in Edman and Nilsson 1968) The velocity values obtained with a large load correspond to a shorter length of the contractile component than do the values obtained with a small load This is evident from the fact that the contractile unit must undergo a greater amount of shortening before a heavier load can be lifted This factor will to some extent counteract the distorsion of the force velocity curve that is caused by variability in time of collection of the expermental data

II *Force velocity curves derived from quick release measurements using different degrees of damping*

The relationship between isotonic load and velocity of shortening was determined using the release technique described in section I but varying the degree of damping of the isotonic lever 6 complete expts on different papillary muscles were performed Typical oscilloscope traces from releases initiated at the same tension level during

[2] The active state analysis described previously from this laboratory has been made at a previ[illegible] length of the contractile unit usually the length assumed by the active component at the time of the isometric peak twitch tension The active state curve shown in Fig 2 (insert) [illegible] on results obtained in such experiments This curve does not intend to illustrate the ex[illegible] course of the active state during the actual isometric twitch which involves a [illegible] amount of intrinsic shortening

Fig 4 Oscilloscope traces illustrating quick releases during isometric contr[illegible] papillary muscle using different degrees of damping of lever movements Pan[illegible] movements critically damped Panels C and D Lever movements substantially [illegible] Releases carried out from same tension levels before (A and C) and after [illegible]



ment of isometric peak twitch tension Dashed vertical lines indicate times for collection of force velocity data In C and D an interpolated value of the shortening velocity is used as indicated by solid lines Note that the velocity of shortening during the second shortening phase is lower in the case of underdamping of lever movements This depressant effect is most pronounced when the release is done during the relaxation period (*cf* B and D) Also note the smaller *amount* of shortening in D as compared to B Temperature 28 C Contraction frequency 48 per min Resting length 5 5 m

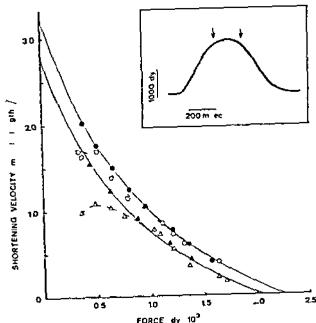


Fig 5 Force velocity curves derived from quick release recordings carried out during the rising and falling phases of the isometric twitch Arrows on top of inserted isometric myogram indicate times for collection of experimental data Filled symbols lever movements were critically damped Open symbols lever movements were underdamped (*cf* Fig 4) Solid lines (referring to filled symbols) have been fitted according to Hill's equation using the following values of the constants a and b
● $a = 1\ 17\ \ 10^3$ dyn $b = 1\ 68$ lengths/sec
▲ $a = 1\ 17\ \ 10^3$ dyn $b = 1\ 57$ lengths/sec
Correlation coefficient between experimental points and data predicted from the hyperbolic curve ● 0 999 ▲ 0 998 Dashed lines have been fitted by eye to data obtained from underdamped releases

the rising and falling phases of an isometric contraction are shown in Fig 4 A—D The greatest amount of damping employed (A and B) was sufficient to reduce the velocity oscillations after the release to ±0 1 lengths/sec (This degree of damping is referred to as *critical damping*) The release record however still exhibited two distinct phases With the lower degree of damping (C and D) there was an overshoot of the initial shortening phase and marked fluctuations in shortening velocity were exhibited during the second phase

The speed of shortening of the contractile component (displayed by the second phase of the isotonic myogram) was depressed when the release movement was insufficiently damped The depressant effect was relatively small when the release occurred early during the contraction period (*cf* A and C in Fig 4) It was much more pronounced however when the release was made after the isometric tension had started to decay (*cf* B and D in Fig 4) It is of great interest to note that the depressant effect appeared even in the absence of any real stretching of the muscle Thus as can be seen on scrutinizing the record illustrated in Fig 4 C there was a continuous shortening of the muscle after the release the lever oscillations merely caused fluctuations in the speed of the shortening movement

Force velocity curves plotted from release records obtained with critical damping and under conditions of underdamping of the lever are presented in Fig 5 The analytical data have been collected 14 msec after the release by the method described in section I In those cases where oscillations were present (underdamped movements) an interpolated value of the velocity was used (see straight line

through oscillations in Fig 4 C and D) The arrows on top of the inserted isometric myogram in Fig 5 indicate the time at which the releases were carried out Note that the muscle tension and hence the degree of extension of the series elastic component was the same at the two instants of release This means that the velocity data obtained refer to virtually the same contractile element length in both situations

The full lines (filled symbols) in Fig 5 are based on release recordings that were carried out with the lever critically damped The two sets of data have been fitted by Hill s equation using the computer analysis described before (see Methods) In both cases there is a very high degree of correlation between the experimental data and the computed hyperbola, the correlation coefficients being 0 999 and 0 998 respectively It should be noted that almost the same values of the constants *a* and *b* in Hill s equation apply at the two different times investigated during the contraction period

The outcome of the analysis is very much different however when the movements of the isotonic lever are underdamped to an extent that the shortening velocity is depressed by the release procedure (dashed curves and open symbols in Fig 5) The depressant effect is progressively larger the lighter the load on the muscle resulting in an upwards convex shape of the force velocity curve The distortion of the force velocity curve is more pronounced when the analysis is performed during relaxation than during the rising phase of the contraction In the curve determined during relaxation (open triangles in Fig 5) maximum speed of shortening can be seen to occur at an intermediate load rather than at the lightest load used Even more pronounced distortions of the force velocity curves than illustrated in Fig 5 (and also greater uncertainty of the analysis) may be produced by further underdamping of the lever movements

Discussion

Distortion of force velocity curve as a result of underdamping of release movements

Quick release techniques have been utilized in several previous studies as a means to analyze the instantaneous relationship between force and velocity of shortening in isolated myocardial preparations The outcome of such analyses has varied greatly and no definite opinion has been formed as to the true shape of the force velocity curve in heart muscle Edman and Nilsson (1968) concluded that the force velocity curve has a hyperbolic shape provided that the data used for construction of the curve are collected at the same instant during the twitch period and at a very nearly constant length of the muscle Brady (1965) Sonnenblick (1967) and Noble Bowen and Hefner (1969) on the other hand found that the curve was markedly different from a hyperbolic function The velocities of shortening at small loads were found by these authors to be appreciably lower than expected from a hyperbolic equation that was fitted to data obtained at intermediate and large loads

The present study provides a reasonable explanation of the discrepant results

obtained in previous experiments by demonstrating the importance of having the lever system critically damped during the release recordings. If the lever movements are sufficiently damped to prevent oscillations from occurring after the release the force velocity data collected at any given time during contraction are fitted well by Hill's equation. The correlation coefficient between experimental points and data predicted from Hill's equation is found to be very high (> 0.99). By contrast if the lever movements are underdamped the velocities recorded at low load become markedly depressed. By using different degrees of underdamping it is indeed possible to reproduce the various non hyperbolic curve shapes presented in previous studies. As no measures were taken to achieve critical damping of the lever movements in the quick release experiments of Brady (1965), Sonnenblick (1967) and Noble, Bowen and Hefner (1969) the results obtained in these studies were probably influenced by the effects discussed here.

The reduction in shortening velocity that occurs in response to an underdamped release clearly reflects a depression of the contractile activity. This is indicated by the fact that the *extent* of shortening against a given load is also reduced after an underdamped release (Fig. 4 D). The depression of contractility is most likely attributable to the inertial oscillations that are imposed on the muscle length when the lever movements are inadequately damped. The speed and amplitude of these length changes are both increased when the muscle is released against successively smaller loads. (This is due to the fact that the speed of the lever movement during the initial shortening phase will be larger in this case leading to a greater kinetic energy of the lever.) On this basis then a more and more pronounced depression of the mechanical activity would be anticipated when going to lighter loads as in fact has been demonstrated in the present study.

The precise mechanism of the deactivating effect is still unclear. The oscillatory movement begins as an overshoot of the initial shortening phase after release. The kinetic energy of the lever built up during the initial step modulates the subsequent shortening movement of the contractile component as the inertial force is alternately added to or subtracted from the isotonic load. Deactivation of cardiac muscle has previously been found to occur in response to quick stretch (Abbott and Mommaerts 1959, Brady 1966) and active shortening ("movement effect") (Edman and Nilsson 1971). However neither of these interventions have been employed to produce the depressant effect recorded in this study. The amount of active shortening that occurs during the initial phase of an underdamped release would be too small to have any appreciable effect upon the mechanical activity. It is furthermore evident (p. 8) that the muscle need not actually be stretched by the inertial lever movements in order to become deactivated. Taken together the results would thus seem to indicate that the mere *changes* in shortening velocity (referred to as accelerations and decelerations) that arise in response to an underdamped quick release are sufficient to cause deactivation of the contractile system in cardiac muscle.

In deciding whether acceleration or deceleration is critical for depression of the mechanical activity it is of interest to consider in some detail the contractile events

that occur after a damped release It is evident that under conditions of adequate damping there is a marked velocity transient acceleration during the initial phase after the release This is due to the fact that the load felt by the contractile unit is suddenly reduced as the series elastic component is slackened In contrast to the situation of an underdamped release however no substantial deceleration occurs at the transition to the second phase as the load remains almost constant in this case The fact that the force velocity curve exhibits a true hyperbolic shape when the data are obtained from damped release recordings strongly suggests that the large acceleration associated with the release movement does not induce any depression of the mechanical activity A depressant effect if it existed would show up as a distortion of the curve at low loads (*cf* above)

On the basis of these findings it would appear that the contractile process is not depressed by accelerations The depressant effect produced by an underdamped quick release is therefore probably to be attributed to the deceleration caused by the inertial oscillation of the lever It should be pointed out that this kind of intervention might in fact be similar to stretching the contractile component during isometric contraction which has previously been shown to depress contractility of heart muscle

The deactivating effect produced by passive stretch (Brady 1966) and active shortening (movement effect) (Edman 1971 Edman and Kiessling 1971 Edman and Nilsson 1971) has been found to depend upon the time during the contraction period at which the movement occurs In accordance with these observations the distortion of the force velocity curve which results from inadequate damping of the lever system becomes more exaggerated as the release analysis is carried out at a later time during contraction (Fig 5) This finding may be an indication that the depressant effects that are produced by these different interventions are all interrelated and involve the same basic change of the contractile machinery (Edman 1971)

Kinetics of V_{max}

The results have confirmed our previous finding (Edman and Nilsson 1968) that V_{max} and P_0 both undergo gradual changes during myocardial contraction such that the ratio of V_{max} to P_0 remains very nearly constant It is essential to point out however that the experimentally derived value for V_{max} (obtained by extrapolation to zero load) may not represent a fully unloaded contractile system (also see Edman and Nilsson 1969) The possibility exists that even in the total absence of external load there may be a substantial intrinsic load acting upon the myofilaments Such a load (which is to be distinguished from the braking force that is inherent in the cross bridge mechanism in Huxley s (1957) model) could be produced by mechanical hindrance of the sliding movement within the myofilament lattice This would be particularly likely to occur at sarcomere lengths below 2 0 μm in which case the I filaments have to pass through the M line structure and moreover the myofilaments are more tightly packed due to double overlap of the thin filaments The

presence of connective tissue in the muscle and the existence of an outer endothelial layer in the preparation may provide another origin of an intrinsic load as both these factors are likely to restrict a widening of the fibres during shortening. A restrain of the lateral expansion of the fibres during shortening would cause a rise in pressure within the fibre (also see Gordon Huxley and Julian 1966) This would produce a longitudinal force at each end of the fibre which would act as a load upon the contractile component

Until the magnitude of the intrinsic load can be assessed it is not feasible to draw any quantitative conclusions as to the speed of shortening of the completely unloaded contractile system As far as the *external* performance of the heart muscle is concerned however the damped release analysis would seem to make clear that the extrapolated V_{max} is not constant It varies with time during the contraction period and in response to inotropic interventions of the myocardium (Edman and Nilsson 1969)

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Note added in proof

A paper has recently been published (Meiss R A and E H Sonnenblick Amer J Physiol 1972 222 630—639) in which it is claimed that the force velocity curves obtained in our previous work were distorted by deactivation that was induced by quick release In fact these data were obtained not by the standard quick release method but by the *damped release technique* developed by us The present paper shows clearly that there is no detectable deactivation when this method is used

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Effects of 2,-4 Dinitrophenol on Release and Uptake of Noradrenaline in Guinea Pig Heart

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Abstract

CHANG P U S von EULER and F LISHAJKO *Effects of 2 -4 dinitrophenol on release and uptake of noradrenaline in guinea pig heart* Acta physiol scand 1972 85 501—505

Dinitrophenol (DNP) was shown to have cardiac stimulant action on the isolated perfused guinea pig heart The initial large increase in the force of contraction of the heart was followed by a smaller response which persisted throughout the DNP perfusion On washout the activity of the heart returned to its previous level Further DNP perfusion had no effect on the heart even though its amine level was not depleted However the positive inotropic effect of DNP reappeared in the heart after perfusion with noradrenaline (NA) Propranolol blocked the cardiac response to DNP The metabolic uncoupler reduced the NA content in the heart and raised the amine level in the perfusate The uptake of exogenous NA and of ^{14}C-NA by the heart was not affected by low and high concentrations of DNP The results suggest that DNP may have a direct action releasing NA from an extragranular pool

DNP uncouples oxidative phosphorylation by interfering with mitochondrial activity resulting in the reduction of ATP (Lehninger Carofoli and Rossi 1967) Presumably as a result of the interference with energy metabolism uptake and retention of noradrenaline (NA) is reduced in adrenal medullary granules (Carlsson Hillarp and Waldeck 1963) and in isolated nerve granules (Euler and Lishajko 1963 1969) and in other isolated tissues including the atria (Wakade and Furchgott 1966) Thus it appears that amine uptake or reuptake in adrenergic nerves is an energy dependent process which is probably linked to an electron transport system (Euler and Lishajko 1969)

This paper describes the effects of DNP on the release and uptake of NA in isolated perfused guinea pig heart and on myocardial contractility

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Methods

Guinea pigs weighing 500—700 g were used The animals were sacrificed by a blow on the head and the heart was quickly set up and perfused with Tyrode solution according to Langendorff The perfusion fluid was aerated with 6 5 % CO_2 in O_2 and maintained at 37 C The composition of the Tyrode solution was as follows (in mmol/l) NaCl 137 KCl 2 7 $CaCl_2$ 1 8 $MgCl_2$ 2 1 $NaHCO_3$ 11 9 glucose 5 6 The perfusion pressure was kept at about 70 cm of water and the force of contraction was measured according to the method of Åstrom Bernhoff and Persson (1970) using a water filled balloon inserted into the right ventricle through the pulmonary artery The recordings were made on a Grass Polygraph (Model 7) The heart was allowed to stabilize for 30 min before the experiments commenced

The heart was perfused with DNP for 30 min unless otherwise stated and at the end of the perfusion the heart was removed blotted and weighed NA was extracted with trichloroacetic acid (TCA) and estimated fluorimetrically by the method of Euler and Lishajko (1961) Perfusate was collected on ice in a small volume of TCA and NA determined as above The perfusate was collected for 5 min at the onset of the action of DNP on the heart Radioactivity was measured in a Packard scintillation spectrometer

The drugs used were (—) noradrenaline bitartrate (Sigma) propranolol hydrochloride (ICI) and dinitrophenol (Merck) (—)^{14}C noradrenaline (57 m Ci/mmol Amersham) was added to the perfusion solution to give a final activity of 4×10^{6} dpm The drugs were dissolved in Tyrode solution immediately before use The concentrations referred to in the text are in terms of the free bases

Results

Effects of DNP on contraction force and noradrenaline release

Force of contraction

Dinitrophenol (DNP) giving final perfusion concentrations ranging from 8×10^{-7} to 4×10 M increased the force of contraction without having any apparent toxic effect on the heart At 8×10^{-6} M (used throughout the investigation unless otherwise stated) there was a large positive inotropic response lasting 4—5 min followed by a smaller response which persisted throughout the perfusion On washout the activity of the heart returned to its previous level On repeated DNP perfusion there was no increase in the force of contraction However the cardiac response to DNP was obtained again after the heart had been perfused with NA (2×10^{-7} M) for 30 min and returned to its previous activity level The results are shown in Fig 1 The positive inotropic response to DNP was abolished by propranolol at a concentration (2×10^{-6} M) which blocked the cardiac stimulant effects of 0 1 μg of adrenaline and noradrenaline

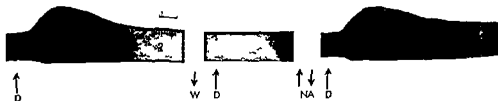


Fig 1 Inotropic response of isolated perfused guinea pig heart to dinitrophenol (D) At D the heart was perfused with 8×10^{-6} M causing a temporary increase in the force of contraction After washout with Tyrode (W) same perfusion with D had no effect but after treatment with NA (2×10^{-7} M at NA) the positive inotropic response to D perfusion was restored

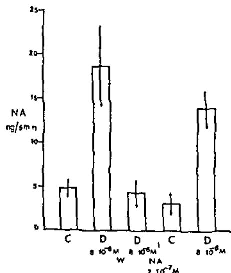


Fig 2

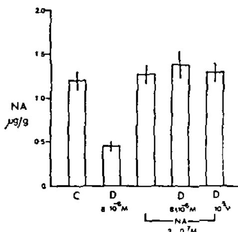


Fig 3

Fig 2 Effect of dinitrophenol (D) on NA content in perfusate from isolated guinea pig heart. The columns represent the mean amine level in perfusate and the bars refer to S E After washout at W succeeding perfusion with D did not cause a rise in the amine level as it did previously After treatment with noradrenaline at NA and return of amine level to normal (C) perfusion with DNP again caused increased NA level in perfusate (D)

Fig 3 Effect of dinitrophenol (D) on NA uptake in perfused and partially depleted guinea pig heart The columns represent the mean amount of NA in the heart and the bars refer to standard error of the mean (S E) Noradrenaline (NA) was given alone or in combination with D The mean amine content in normal heart is indicated by C

Release of noradrenaline

The NA content in heart perfused with Tyrode solution alone for 30 min was 0.88 ± 0.07 μg/g and for 60 min 0.79 ± 0.05 μg/g ($\pm$S E)

DNP perfusion reduced the myocardial NA content significantly after 30 min (P $<$ 0 001) from 1 2 to 0 62 μg/g tissue (Table I) The amine content was further reduced to 0 42 μg/g tissue when DNP perfusion was increased to 60 min

TABLE I Effect of DNP on myocardial noradrenaline content

Mean content $\pm$ S E of mean in μg/g tissue		
Control	Heart perfused with DNP for	
	30 min	60 min
1 2 $\pm$ 0 ? (5)	0 62 $\pm$ 0 08 (4)	0 42 $\pm$ 0 0? (4)

* Significance of difference (t test) compared with the control value (P < 0 001) Figures in parentheses refer to number of hearts

As it can be seen from Fig. 2 the NA in the perfusate was also significantly increased by DNP from 4.9 to 18.9 ng/5 min ($p < 0.001$). The amount of NA released by succeeding DNP perfusion was comparable to the control, being 4.4 ng/5 min. However, after the heart had been previously perfused with NA (2×10^{-7} M) for 30 min and when its cardiac effects had worn off (also indicated by low perfusate NA being 3.3 ng/5 min), DNP perfusion again caused a significant rise in perfusate NA raising it to 14.0 ng/5 min ($p < 0.001$).

Effect of DNP on noradrenaline uptake

After previous lowering of the NA content in the heart perfused with DNP for 60 min from 1.2 ± 0.2 to 0.42 ± 0.02 μg/g tissue, subsequent perfusion with NA (2×10^{-7} M) for 30 min almost completely restored the amine content. When both DNP and NA were perfused together for 30 min, the uptake of amine remained unchanged (Fig. 3). Similar results were obtained when the DNP concentration was raised to 4×10^{-5} M. Thus, the uptake of NA by the heart was not prevented by DNP.

Incorporation of ^{14}C-NA was studied under similar experimental conditions. In two hearts addition of DNP to ^{14}C-NA and NA did not alter the degree of ^{14}C incorporation into the myocardial NA pool. The radioactivity in the treated heart was 842 ± 33 compared with the control value of 740 ± 44 cpm/g tissue.

Discussion

The rise in the amine level in the perfusate following addition of DNP to the perfusing fluid (Fig. 2) indicates a release of NA which probably accounts for the cardiac stimulant effect. This release is accompanied by a fall in the NA content of the heart (Table I). Euler and Lishajko (1969) observed an increased rate of NA release with DNP in isolated nerve granules. In the present experiments, however, continued or repeated DNP perfusion did not release NA or produce any effect on the heart even when its amine content is still considerable (Table I). It therefore appears that the effect depends on NA release from sites which are easily depleted but may be readily reloaded by exogenous NA. These storage sites may correspond to the hypothetical extragranular or "available" pool which functions as an immediate source of transmitter release. It is conceivable that in the presence of DNP this small pool is not refilled from the main granular stores as efficiently as it would normally.

According to Wakade and Furchgott (1966) DNP at comparable concentrations inhibited axonal membrane uptake of NA in electrically driven guinea pig atria. There was also inhibition of amine uptake in perfused spleen (Kirpekar and Wakade 1968) and in isolated nerve granules (Euler and Lishajko 1969). In these findings inhibition of uptake as a result of interference of energy metabolism is thought to be the main mechanism by which DNP releases NA. Although there is no simple

way to account fully for the release of NA by DNP from the isolated perfused heart it is possible that the metabolic uncoupler may have a direct action of transmitter stores located extragranularly at the axon membrane or the juxta membrane level

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Acta physiol scand 1972 85 506—510

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The Fine Structure of the Paracervical (Frankenhauser) Ganglion of the Rat after Permanganate Fixation

By

ANTTI HERVONEN LASSE KANERVA and HEIKKI TERÄVAINEN

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Abstract

HERVONEN A L KANERVA and H TERÄVÄINEN *The fine structure of the paracervical (Frankenhauser) ganglion of the rat after permanganate fixation* Acta physiol scand 1972 85 506—510

The fine structure of the paracervical ganglion was studied in the rat after 3 % $KMnO_4$ fixation Three types of neuronal cells were observed (1) The majority of cells were devoid of dense core vesicles (DCV) (2) The minority of cells with typical neuronal structure contained small (500 Å) and large (1000 Å) DCV (3) The cells resembling those of the adrenal medulla contained vesicles up to 3000 Å in diameter with irregular and dense core The synaptic axon terminals to the different cell types contained agranular vesicles Because no small DCVs were found these synapses are presumably not adrenergic

Though fluorescing adrenergic nerves have been reported around the neurons of the paracervical ganglion of the cat (Rosengren and Sjoberg 1967) the guinea pig (Furness and Malmfors 1971) and human uterus (Owman *et al* 1967) it was not possible to demonstrate in the rat axon terminals with dense cored vesicles of 200—600 Å range by glutaraldehyde fixation followed by postosmification (Kanerva and Teravainen 1972) Synaptic nerve endings of this type are believed to be adrenergic (*cf* Grillo 1966) Recent evidence indicates that the granule within the adrenergic vesicles can be relatively selectively stained by potassium permanganate (Hokfelt and Jonsson 1968) This method was used in the present work mainly to try to demonstrate the extent of adrenergic innervation of the different neurons in the paracervical ganglion

Material and Methods

Altogether 12 adult Sprague Dawley rats were used in the present work 6 of the rats were killed by cutting the neck The paracervical ganglia at both sides of the uterus were immediately removed and fixed by immersion at 4 C for 45 to 60 min in 3 % $KMnO_4$ buffered with Krebs Ringer glucose to pH 7 0 or 0 1 M phosphate to pH 7 0 (Richardson 1966 Hokfelt and Jonsson 1968) The success of the fixation was controlled from the superior cervical ganglion fixed with the same fixative s n its staining properties have previously been

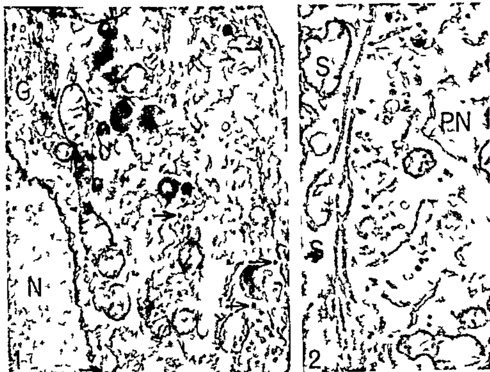


Fig 1 Paracervical ganglion A principal neuron with small dense cored vesicles (DCV arrow) about 500 Å in diameter and large DCV (crossed arrow) about 1000 Å in diameter Nucleus (N) Golgi apparatus (G) ×27 100

Fig 2 Superior cervical ganglion A typical neuron (PN) with many small DCV Satellite cell (S) ×27 100

reported (Hokfelt 1969) Fixation was carried out by perfusion with the same fixatives for 15 min on 6 of the rats under barbiturate anesthesia (Nembutal® 50 mg/kg i w i p Abbott) followed by immersion for 30 to 45 min at 4 C using the perfusion technique previously described in detail (Rechardt 1969 modified by Kanerva and Teravainen 1972) Two animals of both the series were pretreated with nialamide (Niamid® 100 mg/kg i w i p Ciba) 4 h before killing followed by noradrenaline in 2 doses 3 h (1 5 mg/kg b w i p) and 1/2 h (1.5 mg/kg b w i p) before death in order to increase catecholamine concentration of the tissue

Results and Discussion

Depending on the size and shape of dense core vesicles within the neuronal perikarya the neurons (principal cells Kanerva and Teravainen 1972) of the paracervical ganglion can be divided into 3 types The majority of the neurons are devoid of dense core vesicles (DCV) It was earlier assumed that this type of neuron in the superior cervical ganglion might correspond to cells with high acetylcholinesterase activity (Hokfelt 1969) In the cytoplasm of the second type of neuron both small (diameter about 500 Å) and large (diameter about 1000 Å) are present (Fig 1)

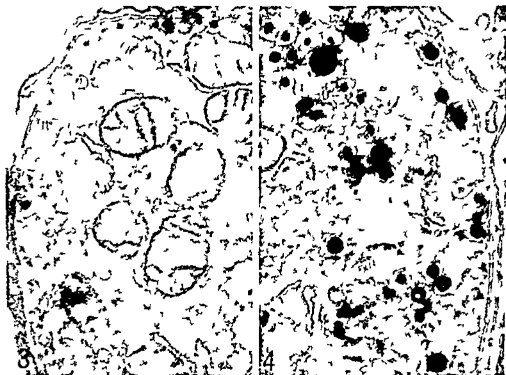


Fig 3 Paracervical ganglion A small granule-containing cell (SGC-cell) with granulated vesicles (GV) mainly 800—1400 Å in diameter ×40 000

Fig 4 Paracervical ganglion A SCG cell with GV also 2000—3000 Å in diameter ×45 000

These are believed to correspond to the cells with noradrenaline fluorescence (adrenergic) since the granules of the vesicle react with $KMnO_4$ (Hokfelt and Jonsson 1968 Hokfelt 1969) In addition to these 2 types of neuron small modified nerve cells structurally resembling those of the adrenal medulla are present in the paracervical ganglion These small granule containing cells (SGC cells) contain numerous cytoplasmic granules which stain heavily with the $KMnO_4$ fixative (Fig 3 4) indicating that they contain amines (Hokfelt and Jonsson 1968) Most of the SGC cells contain granulated vesicles 800—1400 Å in diameter (Fig 3) but in some of the cells larger vesicles up to 2000—3000 Å are present (Fig 4) as previously reported (Kanerva and Teravainen 1972) The granules inside both vesicle types react with the permanganate (Fig 3 4)

Thus there are 2 types of cells containing cytoplasmic vesicles with granules reactive with $KMnO_4$ the SGC-cells and those with the ordinary structure of a neuron This corresponds well to the evidence obtained from fluorescence microscopy which shows both intensely fluorescent small cells (SGC cells SIF cells) and neurons with moderate greenish yellow catecholamine fluorescence in the paracervical ganglion of the rat (Kanerva 1971)



Fig 5 Paracervical ganglion Axon terminal (AT) synapsing with three dendritic extrusions (D) of a principal neuron (PN) which is devoid of DCV The axon terminal contain small and large (arrow) agranular vesicles. × 28 400

Fig 6 Paracervical ganglion Axon terminal (AT) with small and large (crossed arrow) agranular vesicles close to probable dendrites (D) of principal neuron contain no small (arrow) and large (double arrow) DCV Below a SGC-cell × 31 000

The synaptic axon terminals (Fig 5 6) to these different cells contain agranular synaptic vesicles but are devoid of small DCVs about 500 Å in diameter and thu not structured like an adrenergic synap e (Grillo 1966) Many of the axon terminals contain larger vesicles of about 1000 Å with a dense core (Kanerva and Teravainen 1972) which do not react with $KMnO_4$ (Fig 5) Their significance remains to be seen Small DCVs of 200—600 Å were regularly seen in the nerve processes within the ganglion and inside the synapses of the perivascular axons around the ganglion and in many synap e of the superior cervical ganglion excluding any unsuccessful staining reactions Nialamide pretreatment followed by noradrenaline injections in order to increa e the amine content of the vesicles did not p ove successful in in creasing their amounts

There is good evidence that the granules of the small DCVs of about 500 Å seen especially after $KMnO_4$ treatment result from a precipitate from a reaction between $KMnO_4$ and a catecholamine (Hökfelt and Jonsson 1968) The presence of small DCVs has been used to indicate an adrenergic synapse That no synapses of this type to the principal neurons were found was unexpected since the sympathetic nerves which are preganglionic to the neuron of the paracervical ganglion were expected to synapse in the ganglia of the pelvic viscera (*cf* Marshall 1970) It canno

totally excluded that this type of synapse may exist in small quantities because of difficulties in studying large numbers of successive sections by electron microscope However their amount cannot be large It is possible that the $KMnO_4$ method is not absolute in the demonstration of adrenergic nerve terminals since degenerative synapses to the principal neurons have been observed after division of the lumbar sympathetic nerves (Mustonen and Terävainen 1971) and because impulse transmission from the lumbar sympathetic nerves to the uterus can be inhibited by the use of ganglionic blocking agents (Bower 1966) However it is more likely that the cell bodies of the sympathetic preganglionic nerve fibres synapsing in the paracervical ganglion are in the spinal cord This would imply that their axons pass the sympathetic paravertebral ganglia and that the synapses would therefore by analogy (*cf* Volle and Hancock 1970) be cholinergic in function

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Acta physiol scand 1972 85 511—516

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Cholecystokinetic Effect and Concentration of Cyclic AMP in Gall-bladder Muscle in vitro

By

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Abstract

ANDERSSON K E R ANDERSSON and P HEDNER *Cholecystokinetic effect and concentration of cyclic AMP in gall bladder muscle in vitro* Acta physiol scand 1972 *85* 511—516

Contractions were induced by the synthetic C terminal octapeptide of cholecystokinin (C8-CCK) in strips of guinea pig gall bladder in vitro These contractions were relaxed by cyclic AMP (cAMP) and by agents known to increase the intracellular content of cAMP (isoprenaline and theophyllamine) The contractile response to C8 CCK was preceded by an almost 50 per cent activation of the phosphodiesterase activity and a 67 per cent decrease of the cAMP content in the muscle It is concluded that C8 CCK lowers the content of cAMP in the guinea pig gall bladder muscle by activating phosphodiesterase This metabolic response to C8-CCK may be of importance for the development of the contractile activity

Cholecystokinin (CCK) is known to have several different effects it contracts the gall bladder and relaxes the sphincter of Oddi by a direct effect on the smooth muscle cells it stimulates the propulsive peristalsis in the small intestine by an effect mediated by a cholinergic nervous pathway (Hedner and Rorsman 1968 1969) and it stimulates the exocrine pancreas to produce a secretion rich in enzymes (Jorpes and Mutt 1966) All these actions are exerted by the same part of the CCK molecule its C terminal octapeptide (Mutt and Jorpes 1968 Hedner 1970) Because CCK in low concentrations produces such a variety of effects a common mechanism of action on the cells in the different target organs could be expected

The effect of CCK on the exocrine pancreas has been shown to be mediated by an increase of cyclic adenosine 3 5 monophosphate (cAMP) in the glandular cells (Kulka and Sternlicht 1968) cAMP is also supposed to be one of the factors influencing contraction and relaxation of smooth muscle cells (Bueding *et al* 1966 Andersson *et al* 1972) The contractile response to CCK in rabbit gall bladder has been suggested by Amer (1969) to be dependent on a decrease of the tissue level of cAMP by an activation of phosphodiesterase (PDE) However no direct evidence for such a mechanism has been provided Therefore in the present study the contractile response of the isolated guinea pig gall bladder to the synthetic C terminal octapeptide of CCK (C8 CCK) was investigated with special regard to changes of the intracellular level of cAMP and of the activity of PDE

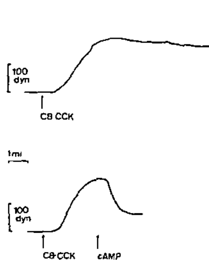


Fig 1

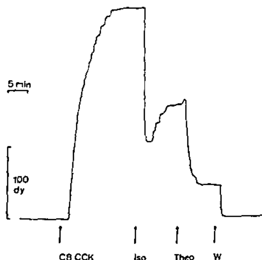


Fig 2

Fig 1 Upper record Effect of C8 CCK (2×10^{9} g/ml) on the tension of a gall bladder strip Lower record Relaxation of a contraction elicited by C8 CCK (2×10^{9} g/ml) in the same preparation after the administration of cAMP (1×10^{3} g/ml)

Fig 2 Effect of isoprenaline (Iso 16×10^{6} g/ml) and theophyllamine (Theo 2×10^{4} g/ml) on gall bladder strip contraction elicited by C8 CCK ($1\ 2 \times 10^{9}$ g/ml) W = washing out the drugs

Material and methods

0 guinea pigs of either sex weighing 200—300 g were used The animals were killed by a blow on the head and the gall bladder was dissected out The fundus and cysticus parts were cut off and the ring thus obtained was cut open to get a strip of gall bladder tissue The strip was mounted in an organ bath containing 25 ml Krebs solution bubbled with a mixture of 95 % O and 5 % CO The temperature was kept constant at 37 C Isometric tension was recorded by means of force transducers (Statham FT 03 Swema SG 4—45) on different recorders (Heathkit Heath Company Servogor 2 type RE 520 Goertz Electro Grass polygraph model 5)

In the experiments designed for determinations of the activity of PDE and content of cAMP each gall bladder strip was cut longitudinally into 3 pieces The mucosa was removed mechanically under a dissection microscope and the pieces were weighed on a torsion balance Each piece was then mounted in an organ bath and connected to a transducer Thus the same gall bladder could furnish material for determinations at 0 20 and 180 s after the administration of C8 CCK The activity of PDE was measured according to Kukovetz and Poch (1970) cAMP was determined according to Kakiuchi and Rall (1968)

C8 CCK the synthetic C terminal octapeptide of CCK was obtained as a gift from Dr M A Ondetti The Squibb Institute for Medical Research New Brunswick U S A

Results

In most preparations submaximum contractions were obtained with $1—10 \times 10^{9}$ g/ml of C8 CCK in the bath Compared with the effect of acetylcholine ($4—20 \times 10^{8}$ g/ml) the response to C8 CCK apepared after a longer latency period and the contraction developed slower Th e differences were more pronounced after low doses of the drugs

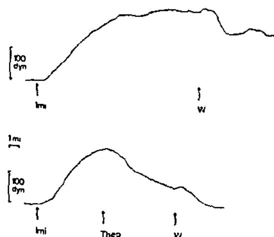


Fig 3

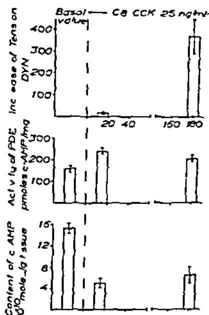


Fig 4

Fig 3 Upper record Effect of imidazole (Imi 3×10^{-3} g/ml) on tension of gall bladder strip Lower record Relaxation by theophyllamine (Theo 2×10^{-4} g/ml) of a contraction induced by imidazole (Imi 3×10^{-3} g/ml) in the same preparation W washing out the drugs

Fig 4 Changes of tension activity of PDE and concentration of cAMP in gall bladder muscle after the administration of C8 CCK 2.5×10^{-8} g/ml Mean±S E n = 5

The response to C8 CCK was not affected by pretreatment of the preparations with phenoxybenzamine or propranolol (1×10^{-6} g/ml) for 15 min

When a response to C8 CCK was fully developed it always persisted without any appreciable decrease in tension for more than 10 min (Fig 1) The plateau of increased tension permitted testing of the relaxing effects of different substances

As can be seen from Fig 2 the administration of isoprenaline to a preparation contracted by C8 CCK was followed by a rapid decrease of the tension Theophyllamine caused a further relaxation of the muscle so that the control tension was almost reached Theophyllamine alone in doses of 0 2—1×10^{-3} g/ml invariably relaxed the muscles contracted by C8 CCK. The degree of relaxation was dependant on the dose of theophyllamine After washing out the theophyllamine the response to C8 CCK was restored within a few minutes

cAMP added to the bath to a concentration of 1×10^{-3} g/ml caused a rapid relaxation of contractions elicited by C8 CCK (Fig 1) but dibutyryl cAMP in the same or even higher concentrations had no relaxing effect

Imidazole 3×10^{-3} g/ml invariably produced a submaximum or a maximum contraction of the gall bladder strip These contractions were counteracted by theophyllamine (Fig 3) and by isoprenaline Contractions evoked by acetylcholin

and potassium ions (Krebs solution with NaCl replaced by KCl) could also be relaxed by these agents

The mean basal content of cAMP in the gall bladder muscle was $1\ 53 \pm 0\ 09 \times 10^{9}$ mol/g (n = 5) 20 s after the addition of $2\ 5 \times 10^{8}$ g/ml of C8 CCK and just as the tension started to rise there was a marked increase in the activity of PDE ($8 \pm 2 \times 10^{8}$ mol/g/h of cAMP hydrolyzed, $p < 0\ 02$) and a significant decrease ($1\ 03 \pm 0\ 06 \times 10^{9}$ mol/g $p < 0\ 001$) in the tissue level of cAMP (Fig 4) After 180 s when the increase in tension was more marked the activity of PDE was still increased and the content of cAMP was decreased (Fig 4)

Discussion

There is increasing evidence that contraction and relaxation of smooth muscle is regulated by the intracellular concentration of free calcium ions (Ebashi and Endo 1968 Ruegg 1971) In intestinal smooth muscle cAMP seems able to reduce the free intracellular calcium concentration by stimulating a microsomal calcium binding mechanism and thereby relaxing the muscle (Andersson and Nilsson 1972) It has been demonstrated that contraction of smooth muscle induced by carbacholine (Andersson 1972 a) and by phenylephrine (Andersson 1972 b) was associated with an initial decrease in the cAMP content which was evident just as the muscle started to contract This initial decrease however was followed by an increase in the cAMP concentration related to an inhibition of the PDE activity by calcium ions Such an increase in the intracellular level of cAMP was probably counteracting the contracting action of the calcium ions (Andersson 1972 a) It has also been shown that imidazole which reduces the cAMP content by activation of PDE contracted rabbit colon and potentiated the contracting effect of carbacholine and potassium ions (*Andersson et al 1972*)

Amer (1969) showed that imidazole could induce contraction of the isolated rabbit gall bladder In this preparation he also found that contractions elicited by CCK could be relaxed by added cAMP and by agents known to increase the intra cellular content of cAMP such as theophylline and glucagon On the basis of these findings he suggested that CCK like imidazole might contract the gall bladder muscle by activation of PDE and thereby lower its content of cAMP

The experimental results of Amer (1969) were confirmed in the present investiga tion However without actual determinations of the intracellular levels of cAMP and of the activity of PDE it is not possible to decide whether activation of PDE might be of importance for the initiation of gall bladder contraction by CCK because contractures induced by other agents *e g* acetylcholine and potassium ions could also be relaxed by cAMP and by theophyllamine In rabbit colon such contractures were not associated with any activation of PDE (Andersson *et al* 1972)

In the present investigation it was found that cAMP but not dibutyryl cAMP relaxed the contraction elicited by C8 CCK This difference was somewhat un expected as dibutyryl-cAMP which is supposed to penetrate the cell membrane

more easily than cAMP has been reported to produce a pronounced relaxation of rat ileum (Kawasaki *et al* 1969) On the other hand the present findings are in accordance with the results of Andersson and Mohme Lundholm (1970) showing that in contrast to cAMP its dibutyryl derivative had no relaxing and no metabolic effect in rabbit colon

In different rabbit tissues Amer (1971) was able to demonstrate 2 allosteric forms of PDE One of these forms (PDE I) had a low affinity for cAMP but a high maximum velocity whereas the other (PDE II) had a high affinity but a low velocity and was suggested to be the more active one *in vivo* CCK was found to increase the proportion of PDE II in fundic mucosa

In the present study activation of PDE could also be demonstrated in gall bladder muscle after administration of C8 CCK The contractile response was preceded by an almost 50 per cent increase in the activity of PDE and a decrease of cAMP to less than 33 per cent of the control value These findings provide evidence that C8 CCK really has a metabolic effect similar to that evoked by imidazole and of the same magnitude (Andersson *et al* 1972) This metabolic effect may by itself explain the contractile effect of C8 CCK However other effects of the peptide must also be taken into account Thus Golenhofen *et al* (1971) showed that in the common bile duct administration of CCK produced a depolarization with accelerated spike discharges associated with an increased tension More than one mechanism therefore might be of importance for the initiation of the contractile effects of CCK and of C8 CCK

CCK has been shown to increase the cAMP content of exocrine pancreas cells (Kulka and Sternlicht 1968) and the hormone thus seems able both to increase and to decrease the cAMP content in different cells a double action that the hormone shares with the prostaglandins (Butcher 1970) epinephrine (Robison and Sutherland 1970) and carbacholine (Andersson 1972 b) Whether CCK thereby influences different mechanisms remains to be studied However in the gall bladder adrenergic and cholinergic receptors do not seem to be involved as they can be blocked without affecting the contractile response to C8 CCK and CCK (Hedner and Rorsman 1968 Amer 1969)

The present results point to the possibility that the effect of polypeptides on the intracellular cAMP level can be mediated not only via the adenylcyclase system and cAMP formation but also by an influence on the PDE activity and cAMP degradation However it is not known whether C8 CCK which reasonably cannot penetrate the cell membrane activates PDE directly or via an intermediate link

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Acta physiol scand 1972 85 517—522

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Dipsogenic Effects of Intracarotid Infusions of Various Hyperosmolal Solutions

By

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Abstract

OLSSON, K. *Dipsogenic effects of intracarotid infusions of various hyperosmolal solutions.* Acta physiol scand 1972. *85.* 517—522.

Thirst in response to intracarotid and intravenous infusions (1.5 ml/min) of various hypertonic equi-osmolal solutions was studied in the goat. Intracarotid infusions of 1 M NaCl and of 2 M fructose induced conspicuous cumulative drinking. The amount of water drunk during intracarotid infusions of 2 M urea and glycerol was only about a third of that consumed during the corresponding infusions of NaCl and fructose. During intracarotid infusions of 2 M galactose and glucose drinking was inconsistent. Of the intravenous infusions only hypertonic NaCl had a consistent dipsogenic effect. However, the amount of water consumed was considerably smaller and the latency time for drinking much longer than during the intracarotid infusions of NaCl. It is concluded that intracarotid infusions of hypertonic solutions act as considerably stronger thirst stimuli than corresponding intravenous infusions, and that the most pronounced dipsogenic effect is obtained by intracarotid infusions of those hypertonic solutions which also most effectively release antidiuretic hormone in the hydrated goat. The possibility is discussed that intracarotid infusions may stimulate the thirst mechanism indirectly via a rise in the Na concentration of the cerebrospinal fluid.

A normal water balance depends upon a regulated release of antidiuretic hormone (ADH) from the neurohypophysis and upon an efficient thirst mechanism which ensures that inevitable water losses become compensated. Much evidence has been produced that these two main aspects in the control of water balance are regulated by principally the same cerebral mechanism (*cf.* Wolf 1958). The current idea is that hypothalamic osmoreceptors (sensitive to changes in their own volume) (Verney 1947) are essential for a normal regulation of both ADH release and thirst.

The liberation of ADH in response to intracarotid infusions of various hypertonic solutions was studied recently in the pre-hydrated goat (Eriksson, Fernandez and Olsson 1971). Some results of this study appeared incompatible with the idea of an osmometric regulation of the ADH release. However, no conclusions regarding the thirst mechanism could be drawn since the expts were performed in hydrated animals. Therefore it was of interest to investigate the dipsogenic effects of similar infusions in goats in normal water balance. The results of such a study are present here.

Methods

Animals 5 adult female goats (b wt 30—38 kg) were used The animals were routinely confined in metabolism cages by means of collars All expts were conducted in these cages The goats were fed chopped hay at 8 a.m and received 300 g of commercial grain mix (with 3 g of NaCl added) each afternoon They had free access to water at a temperature of 20±1 C

Implantation of intraarterial catheters and infusion technique Four goats had polyvinyl catheters (ID 0 8 mm OD 1 2 mm) permanently implanted bilaterally into the external carotid artery via its superficial temporal branch For further details see Eriksson *et al* 1971 The catheters were flushed daily with isotonic saline and filled with heparin solution. During the intracarotid infusions the free end of the vascular catheter was connected with a perfusion apparatus ("Unita/Perfusor") via a polyethylene tubing This tubing was taped on to one horn of the animal and was flexibly suspended above the animal by use of a spring balanced wheel In this manner the goats retained their original freedom of movement during the infusions The same technique was used for the intravenous control infusions Here the tubing from the infusion pump was connected to a polyethylene cannula introduced into the jugular vein. All intravascular infusions were made for 1 h periods at a rate of 1 5 ml/min. The infusions were started between 10 and 11 a.m At this time of the day the goats had eaten to satisfaction from the morning refill of the hay bin They had also performed postprandial drinking

Infusions into the 3rd cerebral ventricle One goat had a permanent cannula implanted into the anterior part of the 3rd cerebral ventricle Infusions of 1 M fructose solution were made via this cannula at a rate of 10 μl/min for 30 min periods The implantation and infusion techniques have been described earlier (Andersson, Olsson and Warner 1967)

Blood samples Blood samples were obtained in heparinized syringes via the same kind of jugular vein cannula which was used for the intravenous control infusions In most expts blood samples were taken as follows 1) preinfusion 2) 10 min 3) 30 min, and 4) 55 min after the onset of the 1 h infusion. The samples were obtained from the ipsilateral jugular vein during the intracarotid infusions and from the contralateral vein during the intravenous infusions.

Analyses Plasma osmolality was determined by the freezing point depression method using an "Advanced Instruments Inc osmometer

Results

Unilateral intracarotid infusions

With one exception (sucrose) the same equi-osmolal hypertonic solutions were used as in the previous study of the antidiuretic response to intracarotid infusions in the hydrated goat (Eriksson *et al* 1971) Thus 1 M NaCl and 2 M fructose galactose glucose urea and glycerol were infused at a rate of 1 5 ml/min for 60 min periods Intracarotid infusions of hypertonic sucrose were omitted since (in contrast to all the other infusions) such infusions had been found to distress the animals

The dipsogenic responses were strikingly correlated to the antidiuretic effects previously seen in the hydrated goat The intracarotid infusions of hypertonic *NaCl* and *fructose* (effectively releasing ADH in the hydrated goat) induced conspicuous cumulative drinking (Fig 1 *left* Weaker and inconsistent dipsogenic responses were obtained during the intracarotid infusions of all other hypertonic solutions (having no or doubtful antidiuretic effects in the hydrated goat) The amount of water drunk during the infusions of *urea* and *glycerol* was only about a third of that consumed during corresponding infusions of NaCl and fructose (Fig 1 *left*) During the infusions of *galactose* and *glucose* drinking was inconsistent and only small amounts of water were taken The results of all expts are summarized in Table I As seen in this table there is no correlation between the rise in ipsilateral jugular plasma osmolality and the amount of water consumed during the various

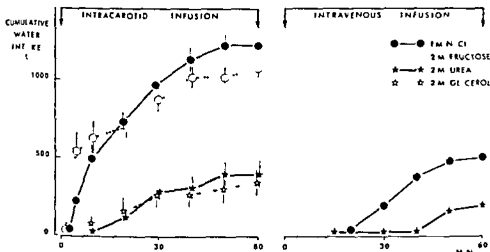


Fig 1 A comparison between dipsogenic responses to intracarotid (left) and intravenous (right) infusions of various hypertonic equi-osmolal solutions in the goat The intracarotid infusions of hypertonic NaCl (n = 18) and fructose (n = 8) caused conspicuous cumulative drinking The corresponding infusions of hypertonic urea (n = 8) and glycerol (n = 7) induced considerably less drinking Intracarotid infusions of equi-osmolal hypertonic solutions of galactose and glucose (not indicated in the figure) had very weak and inconsistent dipsogenic effects Only hypertonic NaCl (n = 10) and urea (n = 6) caused drinking when infused intravenously The latency time for drinking was much prolonged and considerably less water was consumed during the intravenous infusions Values given as means ± S E Number of animals = 4 Rate of infusion = 1 5 ml/min

infusions In fact 55 min after the start of the infusions the lowest ipsilateral plasma osmolality values were seen in the NaCl and the fructose expts Hemodilution due to a large initial water consumption may explain these relatively low values

Bilateral intracarotid infusions

Since the most pronounced dipsogenic effect was obtained with hypertonic NaCl such infusions were used to compare the responses to application in the left and the right carotid artery In two of the goats a marked difference was seen in the

TABLE I Water intake and increase in plasma osmolality in the ipsilateral jugular vein during 60 min intracarotid infusion of various hypertonic equi-osmolal solutions All values are means ± S E Preinfusion plasma osmolality 294 ± 1 mosm kg

Intracarotid inf (1 5 ml/min)	Water intake ml	Increase in plasma osmolality (mosm/kg) Time after onset of infusion		
		10 min	30 min	55 min
1 M NaCl	1220 ± 85 (n = 18)	9 ± 2	11 ± 1	14 ± 2 (n = 12)
2 M fructose	1080 ± 85 (n = 8)	9 ± 1	9 ± 1	1? ± 2 (n = 7)
2 M urea	400 ± 100 (n = 8)	10 ± 2	13 ± 2	16 ± 2 (n = 4)
2 M glycerol	340 ± 110 (n = 7)	9 ± ?	1? ± 1	15 ± 1 (n = 4)
2 M galactose	180 ± 60 (n = 7)	1[illegible] ± 3	14 ± 1	19 ± 3 (n = 4)
2 M glucose	190 ± 90 (n = 7)	10 ± 2	14 ± ?	17 ± 3 (n = 3)

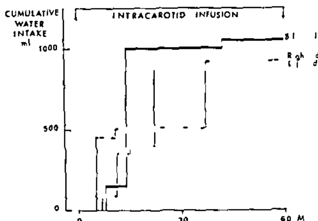


Fig 2 Similar dipsogenic effects obtained by the same load of hypertonic (1 M) NaCl administered simultaneously via both carotid arteries (Bilateral) and via either the right or the left carotid artery in a goat. Rate of infusion 1 5 ml/min (= 2×0 75 ml/min during the bilateral infusion)

dipsogenic response to infusions into the left and into the right artery indicating that the infusions reached the appropriate part of the brain more effectively from one side than from the other In the other two animals the left and right infusions gave very similar responses For this reason the latter animals were chosen for bilateral infusions The 1 M NaCl solution was infused simultaneously into both carotid arteries (rate 2×0 75 ml/min) and the effect obtained was compared to that seen during unilateral intracarotid infusion of 1 M NaCl (rate 1 5 ml/min) As shown Fig 2 the latency time for drinking and the amount of water consumed were very uch the same in the two kinds of expts

travenous control infusions

o ascertain whether the marked dipsogenic effect of the intracarotid infusions of 1 M NaCl and 2 M fructose were elicited centrally intravenous control infusions of these substances were made repeatedly in all animals Similar intravenous infusions of 2 M urea and glycerol also were made because the intracarotid application of these solutions had elicited some drinking (Fig 1 *left*) No intravenous infusions of galactose and glucose were made since the intracarotid application of these substances had caused very little and inconsistent drinking (Table I)

The amount of water consumed during the intravenous infusions of hypertonic *NaCl* was only about a third of that drunk during the corresponding intracarotid infusions The latency time for drinking was also much longer during the intravenous infusions (Fig 1 *right*) Most of the water was drunk during the second half of the infusion period when the blood plasma osmolality had reached approximately the same value (Table II) as that found in the ipsilateral jugular vein during the intracarotid infusions of NaCl

The intravenous infusions of 2 M *fructose* and *glycerol* did not induce drinking and only a slight rise in plasma osmolality occurred during these infusions (Table II) A more pronounced rise in plasma osmolality developed during the intravenous infusions of 2 M *urea* and in 3 out of 6 expts the animals drank some water during the second half of the infusion period (Table II Fig 1 *right*)

TABLE II Water intake and increase in plasma osmolality in the contralateral jugular vein during 60 min intravenous infusions of various hypertonic equi-osmolal solutions All values are mean ± S E Preinfusion plasma osmolality 294 ± 1 mosm/kg

Intravenous inf (1 5 ml min)	Water intake ml	Increase in plasma osmolality (mosm/kg) Time after onset of infusion		
		10 min	30 min	55 min
1 M NaCl	500 ± 70 (n = 10)	5 ± 1	7 ± 1	10 ± ? (n = 5)
2 M fructose	0 (n = 5)	3 ± 2	4 ± 3	4 ± 3 (n = 4)
2 M urea	190 ± 170 (n = 6)	4 ± 3	8 ± 3	11 ± 3 (n = 4)
2 M glycerol	0 (n = 3)	3 ± 0 4	4 ± 1	4 ± 3 (n = 2)

Intraventricular infusions

The goat which had a cannula permanently implanted in the anterior part of the 3rd cerebral ventricle was used for 30 min intraventricular infusions (10 μl/min) of 1 M fructose Expts of this kind were made both in the hydrated and in the non hydrated goat The infusions did not elicit drinking The expts also confirmed the earlier observation (Eriksson *et al* 1971) that the application of hypertonic fructose inside the blood brain barrier does not cause any release of ADH in hydrated goats

Discussion

The expts reported here show a) that intracarotid infusions of hypertonic solutions act as a considerably stronger thirst stimulus than corresponding intravenous infusions and b) that the most pronounced dipsogenic effect is obtained by those hypertonic solutions which also most effectively release ADH in the hydrated animal (Eriksson *et al* 1971) This supports the idea that thirst and ADH release are regulated by principally the same cerebral mechanism However these studies of thirst and ADH release in the goat also have provided results which appear in compatible with the current osmoreceptor theory (Verney 1947 Jewell and Verney 1957)

Most evidence for a hypothalamic osmometric control of the water balance is based on studies in which little attention has been paid to the possible interference of the brain barrier systems An effective blood brain barrier appears to exist for fructose (Crone 1965 a) urea glycerol and Na ions (Yudilevich and de Rose 1971) A barrier also exists for the free exchange of glucose and galactose between the blood and the brain tissue These two monosaccharides are transferred from the blood to the brain by a rate limiting carrier mediated transport mechanism (Crone 1965 b Csaky and Rigot 1968) It seems likely therefore that intracarotid infusions of all these substances in equi osmolal hypertonic solutions should cause approximately the same degree of brain dehydration If so all kinds of intracarotid infusions made in this and the previous study (Eriksson *et al* 1971) ought to have stimulated cerebral osmoreceptors effectively provided the receptors were located inside the blood brain barrier Nevertheless a rise in the carotid blood osmolality

obtained by infusions of hypertonic NaCl and fructose acted as much more potent stimuli to thirst and ADH release than the equivalent rise elicited by infusions of urea glycerol galactose and glucose It seems unlikely also for another reason that thirst and ADH release in response to intracarotid infusions of hypertonic NaCl and fructose reflect the activation of a hypothalamic osmoreceptor mechanism Only one of these two stimuli (hypertonic NaCl) elicits thirst and ADH release when applied inside the blood brain barrier in the hypothalamic region (Andersson Dallman and Olsson 1969)

Recent studies of central sodium angiotensin interaction has led to the suggestion that a possible alternative to osmoreceptors in Verney's (1947) sense would be a receptor system which is situated near the 3rd ventricle and which is influenced by the Na concentration of the cerebrospinal fluid (CSF) (Andersson 1971) It is possible that the intracarotid infusions which effectively elicit thirst and ADH release may stimulate a Na sensitive receptor system of this kind by increasing the Na^+ concentration of the CSF Preliminary expts in the anesthetized goat speak in favour of this possibility It was found that the intracarotid infusion of hypertonic fructose not only increases the osmolality of the CSF but also causes a marked rise in its Na concentration (Olsson 1972) The bilateral infusions showed that a blood borne load of hypertonic NaCl which is divided between both sides of the brain can elicit thirst as effectively as the same load concentrated to one half of the brain (Fig 2) This would be expected if intracarotid infusions of hypertonic NaCl ulate the thirst mechanism indirectly via the CSF

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Acta physiol scand 1972 85 523—531

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Effects on the Pulmonary Circulation of Suddenly Induced Intravascular Aggregation of Blood Platelets

By

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Abstract

Bo G and J Hognestad *Effects on the pulmonary circulation of suddenly induced intravascular aggregation of blood platelets* Acta physiol scand 1972 85 523—531

When intravascular platelet aggregation was induced by intravenous infusion of collagen extract a temporary and marked increase in pulmonary vascular resistance (PVR) was observed The number of circulating platelets was gradually reduced after the first few infusions of collagen. After 4—6 infusions of collagen the vascular response vanished and the platelet count in arterial blood remained fairly stable When a vasodilator was infused simultaneously with the collagen the pressure response could be abolished Histological examinations of lungs at the peak of a pressor response to collagen revealed aggregated platelets in small arterial vessels and capillaries After many infusions larger vessels were found occluded by agregated platelets It is concluded that platelet aggregation in the blood arriving in the lung creates a strong vasoconstriction in the pulmonary vascular bed This response is related to release of material from the platelets

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It has been shown in previous investigations on cats that blood platelets are a prerequisite for the development of high pulmonary vascular resistance during hemorrhagic hypotension Thrombocytopenic animals were protected against the progressive rise in pulmonary vascular resistance (PVR) which developed in animals with a normal number of blood platelets (Bo and Hognestad 1971) From the investigations of Bergentz Lewis and Ljungquist (1971) it is furthermore known that labelled platelets are trapped in the lungs during shock and trauma It has therefore been suggested that the pulmonary hypertension seen subsequent to trauma and hypotension may in some way be caused by aggregated blood platelets and released material

The aim of the present work has been to study the effect on pulmonary vascular resistance of induced intravascular platelet aggregation Platelet aggregation was achieved by intravenous injections of tendon extract Collagen is known to cause platelet aggregation both *in vivo* and *in vitro* Platelets will adhere to collagen fibrils and this will again give rise to a secondary aggregation of other

(Hovig 1963) During aggregation a platelet release reaction will also take place Collagen itself has no effect on smooth muscle cells and the fibrils of the extract are so small that they should not occlude the pulmonary vascular bed

A considerable but transient increase in pulmonary vascular resistance was observed upon injections of collagen The results of the experiments indicate that this response was not caused by physical obstruction of the vascular bed but by contraction of smooth muscle cells in the pulmonary resistance vessels This contraction was apparently directly or reflexly induced by materials released from the platelets

Methods

Animals Cats weighing 2 5—4 kg were used They were anesthetized by intraperitoneal injections (30 mg/kg) of sodium pentobarbitone (Nembutal® Abbott)

Ventilation After tracheostomy a muscle relaxant Alloferine (1/2 mg/kg) was given and positive pressure ventilation started with a piston pump respirator (The Ideal Respiration Pump C F Palmer Ltd London) The respiration frequency was 14 per min With the use of water seals the end tidal pressures were usually kept at 6—8 and 2 cm of water respectively The respirator s tidal volume was adjusted so as to keep the arterial P_{CO} at 30 mm Hg which is the normal level for cats Standardized hyperinflations were carried out carefully at regular intervals and between each test The ratio of the respirator s tidal volume to the inspiratory peak pressure was calculated during and between each test and was used as an expression of the pulmonary compliance

Surgical procedures Pressure and flow recordings The thorax was opened widely by a ernum splitting in ision Catheters of polyethylene were introduced into the femoral artery pulmonary artery and left atrium for recordings of the femoral arterial pressure (P_{FA} with a Statham P23Gb transducer) the pulmonary arterial pressure (P_{PA} with a Statham P23Db transducer) and the left atrial pressure (P_{LA} with a Statham P23De transducer) respectively Infusions were carried out through catheters placed in the femoral veins and with their tips in the caval vein

A flowprobe was placed around the a cending aorta and flow was recorded by a Nycotron square wave flowmeter (type 372 Nycotron A/S Norway) The pressure and flow transducers were connected to a six channel Sanborn recorder (model 320 Sanborn Co California)

The animals were placed on a heated table Postoperatively they were covered by a polyethylene tent into which was led warm moist air The animals bod temperature was thereby maintained at the normal level

Mean pulmonary vascular resistance PVR was calculated according to the formula

$$PVR = \frac{P_{PA} - P_{LA} \text{ (mm Hg)}}{\text{Mean flow (ml/min)}}$$

Percentage changes are given when PVR alterations in different animals are compared

Thrombocyte counts in arterial blood samples were carried out according to the method of Brecher and Cronkite (1950)

pH measurements were done using Radiometer equipment (pH meter 22 equipped with a pH electrode type G 297/G and a gas mixing apparatus) The arterial P_{CO} was calculated by the equilibration method and according to Siggaard Andersen s nomogram A polyethylene catheter was inserted into a femoral vein ith the tip placed in the caval vein

Collagen and bradykinin infusions 0 5 *ml of collagen suspension (prepared* as described by Holmsen 1969) was infused intravenou l in the course of 1 min When the subsequent pressure response had vanished (usually after 10 min) a new infusion was given The infusions were usually repeated until there was no pressure response to a collagen dose

In some experiments bradykinin (Sandoz) was also infused in doses of 80 μg/min For this infusion a Harvard Infusion Pump (model 947 Harvard Apparatus Co Mass) was used Bradykinin infusions were either ca ried out before or simultaneously with the collagen infusions

Histological examination of the lung The animals were killed by an overdose of Nembutal The fixative (Zenker s solution) was th n poured into the bronchial tree so as to prevent collapse of the lungs

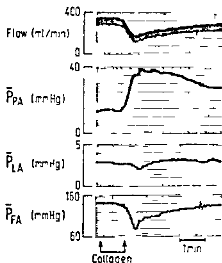


Fig 1 Effect in a cat of 0 5 ml of collagen administered intravenously on mean aortic blood flow mean pulmonary arterial pressure (P_{PA}) mean left atrial pressure (P_{LA}) and mean femoral arterial pressure (P_{FA})

The lungs were then immediately removed and immersed into the fixative Specimens from all lobes of the lung were embedded and sections 4 μ thick were cut

In most animals lung tissue sections were taken when several (6—7) infusions of collagen had been carried out In 3 cats however only one infusion of collagen was given The lungs were then removed at the peak of the subsequent response and sections were taken from the hilus region as well as from peripheral parts of the lung lobes

Results

Intravenous infusion of 0 5 ml of the collagen suspension caused a sharp rise in P_{PA} usually from the mean value of about 15 mm Hg and up to about 35 mm Hg Aortic flow simultaneously decreased by 25 per cent of the resting value This pressure/flow response lasted for 5—7 min Both P_{PA} and flow returned to near initial values within 10 min (Fig 1) The calculated pulmonary vascular resistance (PVR) consequently rose tremendously subsequent to such an infusion the mean rise in 5 animals being almost 500 per cent Great variations were however seen between the individual animals (range 157—859) P_{LA} showed a minute and transient reduction whereas P_{FA} fell more markedly (Fig 1)

TABLE I Percentage rise in PVR in 5 animals upon repeated infusion of collagen

Infusions no	A	B	C	D	E
1	157	500	859	908	669
2	143	416	769	200	669
3	114	942	1102	183	717
4	107	447	663	166	669
5	—	—	—	—	596
6	—	—	—	—	596
7	—	—	—	—	—

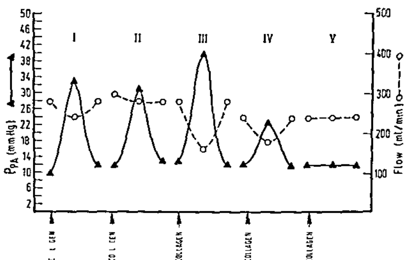


Fig 2 Pulmonary vasopressor responses to repeated infusions of collagen in the same cat. Interval between infusions 10—15 min

Subsequent to the first infusion the number of circulating thrombocytes in arterial blood was found to be reduced by about 20 per cent In some experiments platelet counts in arterial blood were taken both at the top of an infusion response and afterwards A consistently higher count was always obtained after the response rather than during its peak

The ratio of the respirator's tidal volume to the respiratory peak pressure fell during the response to a mean of 60 per cent This ratio returned to its preinfusion level simultaneously with the normalization of flow and pressure

The following trend was then observed (Table I and Fig 2) In 5 animals the first infusion of collagen was followed by 6—7 subsequent infusions The first 4 infusions caused a marked rise in PVR in all the 5 animals Four of the animals did

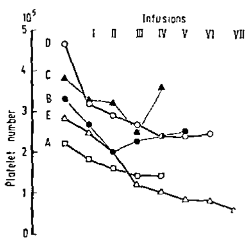


Fig 3 Platelet count in 5 animals during a period of repeated intravenous infusions of collagen

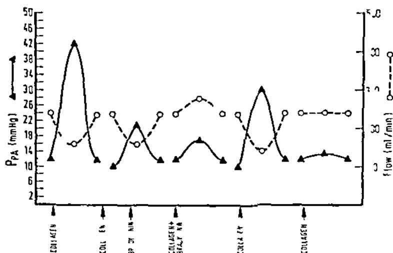


Fig 4 Influence of simultaneous bradykinin infusion on the pulmonary vasopressor response to collagen infusions Collagen response exhausted at infusion no 5

not respond to further infusions One animal (E) responded also to the 5th and the 6th infusions but there was no response to the 7th The platelet count in arterial blood (Fig 3) decreased in all animals up to the 3rd infusion In 4 animals it then seemed to stabilize or even rise moderately In one animal (E) it decreased further

Bradykinin which has been previously found to temporarily reduce the high pulmonary vascular resistance in hypotensive animals were given in connection with the collagen infusions in 2 of the present experiments Bradykinin infusions were either started somewhat before or simultaneously with the collagen infusions (Fig 4) Following one collagen response two subsequent pressor responses to such combined infusion were weak or absent Pure collagen infusion carried out before or just after the combined ones did however give the usual pressure response

In 1 expt the vagal nerve was cut bilaterally between the first and the subsequent infusions of collagen This did not alter the pressure response to collagen in any way

Morphology

The histological picture differed in the two animal groups examined Macroscopically the lungs from both groups showed an almost normal appearance In the first group of animals which had only one infusion of collagen and where the lungs were removed at the peak of the pressor response aggregated platelets were found in small arterioles and capillaries (Fig 5) The aggregates were unevenly distributed in the lung The diameter of the occluded vessels was usually less than 100 μ Some larger arteries contained freely floating clusters of aggregated platelets (Fig 6) Large vessels including the main pulmonary artery showed no aggregates

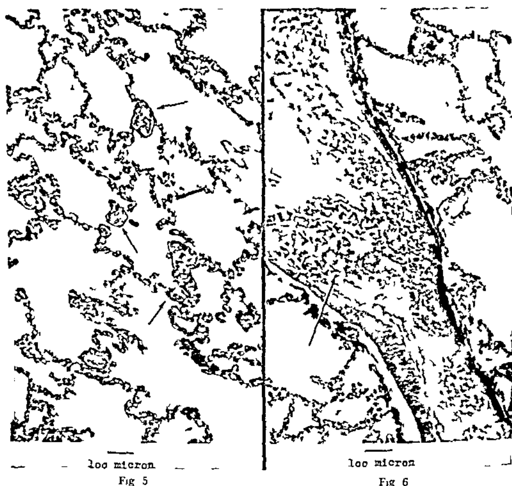


Fig 5 Platelet aggregates in small arterioles and capillaries (arrows) from lung cut out on top of the pressure response

Fig 6 Clusters of aggregates in large vessel from lung cut out on top of the pressure response (arrow)

In the second group of animals which had received several infusions of collagen a different picture was seen Several of the larger vessels with a diameter greater than 100 μ were occluded with apparently densely packed platelets Few smaller vessels were occluded The aggregated platelets showed a varying degree of viscous metamorphosis (Fig 7)

Discussion

Acute pulmonary hypertension may be provoked in a variety of ways One is to cause partial occlusion of the pulmonary vasculature When part of the vascular bed becomes occluded during unchanged cardiac output then flow must increase in

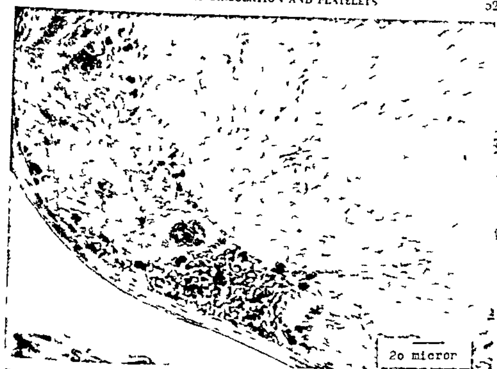


Fig 7 Densely packed platelets occluding large vessel from lung where several infusions of collagen was given Note the varying stages of metamorphosis

the remaining open vessel and there is a tendency towards increase in pressure However the vascular compartment of the lung may be distended to a large extent At constant flow conditions it has been shown that a large fraction of the vasculature must be closed off before there is much rise in the pulmonary arterial pressure Our own experiments on the cat show that when we double the flow through the right lung by clamping the left pulmonary artery PVR in the right lung will decrease by more than 20 per cent

The usual method used to occlude pulmonary vessels is to introduce emboli of a certain size into the pulmonary artery The size of the emboli is of great importance for the pressure/flow response in the lung (Price Hata and Smith 1955) The reason is apparently that a vasoconstrictor reflex mechanism operates when small arteries and arterioles are triggered When only larger arteries are occluded no reflex vasoconstriction will occur Smaller sized particles will cause a pressor response even when moderate quantities are injected (Dexter 1965)

The usual response to collagen infusions in the present experiment was a rise in P_{PA} and a fall in aortic flow Since P_{LA} was fairly constant PVR must have risen considerably The collagen particles themselves were too small to occlude the vessels This was also born out by the responses to late collagen infusion When the presso

response was abolished great amounts of the collagen suspension could be infused *without any influence on flow and pressure However in the experiments where the* lungs were removed at the peak of the first collagen response occluding platelet aggregates were found in small arterial vessels and in capillaries Judging from the accompanying pressor response aggregates in such positions are apparently connected with an element of strong vasoconstriction The effect of physical blockade of some such vessels cannot in itself explain the large increase in PVR.

The experiments with bradykinin infusion give additional support to the presence of a strong vasoconstriction element in the pressor response to collagen Bradykinin which acts on the smooth muscle cells will in animals with normal PVR cause a rise in P_{PA} and also a rise in P_{LA} At the same time the cardiac output shows some *increase consequently PVR is not much changed During pulmonary hypertension* however bradykinin reduce PVR markedly (Bo Hauge and Hognestad in preparation) When bradykinin was infused simultaneously with collagen the pressor response of the latter substance was very much reduced Two components then probably compete on the level of smooth muscle cells namely one or more vasoconstrictor effects from aggregated platelets and the vasodilating effect of bradykinin The number of platelets trapped in the lungs was the same during a combined infusion of bradykinin and collagen We assume therefore that the physical vessel lockade caused by the aggregates was in the same in the 2 series

Aggregated platelets will more or less markedly release their content of serotonin histamine *catecholamines and nucleotides These substances* may exert a vasoconstrictor influence directly on the smooth muscle cells in the lung vessels More over they may stimulate receptive sites in the lungs and cause some reflex vasoconstriction

The present experiments do not allow a differentiation between direct and reflex vasoconstriction effects of released platelet material

During one individual pressure response the lowest platelet count was obtained at the peak of the response Afterwards the count increased somewhat This indicates that some of the platelets are only temporarily trapped in the pulmonary vascular bed Ultrastructural studies on platelets in arterial blood after collagen infusion have indicated that some of the platelets have been involved in a reversible aggregation reaction (Nicolaysen *et al* in preparation)

In the lungs where several collagen infusions had been carried out vessels of larger size were occluded and the aggregates were more densely packed In some of the vessels it appeared that several layers of aggregates were superimposed on each other

A remarkable observation was that the pressor response in the lungs seemed to be exhausted when several collagen infusions had been given The number of platelets in arterial blood did not show any alteration at this point The exhaustion of the response can be explained in two ways 1 The remaining number of platelets may be too small to provoke the response 2 The remaining circulating platelets do not react with collagen

From Table I it appears that the pressure response is rather suddenly abolished This points to the platelets becoming non reactive to collagen which might be a consequence of the repeated stimulations towards platelet release When using different embolizing materials the platelets and their reactions should be taken into account Many of the foreign substances which have been u ed may attract platelet and thereby an aggregation with subsequent release of platelets may contribute to the observed effects on the pulmonary vasculature

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Effects of Chlorpromazine, Imipramine, and Quinidine on the Mechanical Activity of Single Skeletal Muscle Fibres of the Frog

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Abstract

ANDERSSON K E *Effects of chlorpromazine, imipramine and quinidine on the mechanical activity of single skeletal muscle fibres of the frog* Acta physiol scand 1972 *85* 532—546

Chlorpromazine (8×10^{-6}—3×10^{-5} M) imipramine and quinidine (5×10^{-5}—10^{-4} M) ... the relaxation phase of potassium induced contractures in single skeletal muscle of the frog without affecting the maximum tension amplitude These drugs also shifted the curve which relates peak contracture tension to external potassium concentration to lower potassium values and lowered the mechanical threshold This is in contrast to other membrane stabilizers such as tetracaine cocaine and procaine which have been reported to shift the curve to higher potassium values and to increase the mechanical threshold In high concentrations chlorpromazine (10^{-4} M) imipramine and quinidine ($>10^{-3}$ M) produced slowly developing and only partially reversible contractures Similar contractures could be evoked in fibres that were depolarized in isotonic potassium methyl sulphate solution and in fibres that had been soaked for 10—30 min in a calcium free medium The results support the idea that the contractile effects produced by chlorpromazine imipramine and quinidine are mediated by action upon intracellular calcium storing structures

When a muscle fibre is depolarized by electrical stimulation or by a high extracellular potassium concentration an increase of the myoplasmic calcium concentration occurs (for reviews see Sandow 1965 1970) This is mainly attributed to a release of calcium from the sarcoplasmic reticulum but the inflow of calcium through the cell membrane is also increased (Bianchi and Shanes 1959 Weiss and Bianchi 1965) At a sufficient concentration of free intracellular calcium (more than 10^{-7} M) the contractile system is activated and the fibre contracts Relaxation occurs when calcium is resequestered by the sarcoplasmic reticulum (Ebashi 1961)

Calcium plays an important role in the excitation contraction relaxation cycle drugs that influence the regulation of myoplasmic calcium concentration could thus also be expected to influence the tension development and relaxation of a muscle fibre Chlorpromazine imipramine and quinidine have been shown to inhibit the active calcium uptake of isolated sarcoplasmic reticulum (Balzer

Makinose and Hasselbach 1968, Fuchs Gertz and Briggs 1968 Further intact muscle both influx and efflux of calcium through the cell membrane inhibited by these agents (Balzer and Hellenbrecht 1969 Little is known about effects of chlorpromazine and imipramine on the mechanical activity of skeletal muscle The actions of quinidine and of its optical isomer quinine have previously been investigated in whole muscle (Santesson 1892 Harvey 1939 Kraver and George 1951 Lammers and Ritchie 1955 Isaacson and Sandow 1967 Huddart 1971 a b)

To obtain further information on the mechanisms of action of chlorpromazine, imipramine, and quinidine in the excitation contraction coupling the contractile effects of these drugs were investigated in single muscle fibres Particular attention was focused on the time course of relaxation of potassium induced contractures and on the relation between peak contracture tension and extracellular potassium concentration The direct contracture inducing effect obtained with high concentrations of these drugs (Benoit Carpeni and Przybyslawski 1964 Isaacson and Sandow 1967 Balzer and Hellenbrecht 1968 Isaacson Yamaji and Sandow 1970 Huddart 1971 a b) was further investigated

Methods

Procedure Single muscle fibres were dissected from the ventral head of the semitendinosus muscle of *Rana temporaria* either on the day of the experiment or on the preceding evening In the latter case the fibres were stored in a refrigerator (4 C) overnight The fibres were carefully cleaned of connective tissue and debris and small portions of their tendons were left at each end Small rhomboid hooks of stainless steel wire (diameter 0 1 mm) were attached to the tendons The fibres were mounted horizontally in a thermostated Perspex chamber between an adjustable lever and a glass tube attached to the anode peg of an RCA tension transducer The chamber contained 1 2 ml and allowed rapid exchanges of solutions The test solutions were kept in thermostated containers about 100 cm above the chamber to provide driving force and were passed through another thermostated vessel adjacent to the chamber during flushes The flow rate obtained in the chamber was 3—3 5 ml/s For determinations of the exchange rate the chamber was filled with Ringer s solution and then flushed with distilled water The amount of sodium remaining in the chamber after 1—5 s perfusion was determined by means of flame photometry These analyses showed that more than 90 per cent exchange of solution could be achieved within 1—2 s

Recording of fibre tension An RCA 5734 mechano-electric transducer was used Its resonant frequency with the glass tube attached to the anode peg was approximately 500 Hz and it gave a linear response to forces up to 800 dyn The signal from the transducer was displayed both on a direct writing ink jet oscillograph (Mingograf Elema Schonander) and on a Tektronix 502 A oscilloscope The oscilloscope trace was photographed by means of a Cossor oscilloscope camera on 35 mm film (Scientia 135) For recording of slowly developing contractures a potentiometer recorder (Heathkit Heath Company) was also used

Electrical stimulation The fibres were stimulated by passing current through a multi electrode assembly consisting of 6 platinum wires spaced at 2 mm intervals along the fibre perpendicular to the longitudinal axis of the preparation The wires were arranged as alternate anodes and cathodes Square pulses of 0 9 ms duration were used and the stimulus strength was adjusted to ensure that each pair of electrodes gave supramaximum stimulation Tetanic contraction was produced by application of a 1 s train of pulses of a frequency of 30—40 Hz After mounting in the bath the fibre was tetanized occasionally over a period of 1 to 2 h Immediately before the experiment was started the fibre was stimulated at five-minute intervals to produce 3—5 tetanic contraction Only fibres producing completely fused tetani were used for the experiments

Determination of sarcomere length Measurements of the sarcomere length at rest were made by direct microscopy at 800× magnification with a water immersion objective (Zeiss 40× NA 0 75 focal length 4 6 mm working distance 1 6 mm) and an ocular micro

TABLE I Composition of solutions (mM)

Solution	KCl	NaCH SO	CaCl	NaCl	KCH_3SO_4	Na phosphate buffer
Normal Ringer s	2	—	1 80	115 50	—	2 0
10 K	10	96 90	1 80	10 60	—	2 0
16 K	—	89 96	1 80	11 54	16	2 0
20 K	—	88 99	1 80	8 51	20	2 0
30 K	—	83 03	1 80	4 47	30	2 0
40 K	—	75 05	1 80	2 45	40	2 0
80 K	—	37 80	1 50	—	80	2 0
117 5 K	—	0 77	1 03	—	117 5	2 0

as described by Edman and Kiessling (1970) All experiments were performed at sarcomere lengths 2.3 to 2 5 μm

Measurements of membrane potential For intracellular recordings glass capillary electrodes filled with 3 M KCl were used The resistances of the electrodes were between 15 and 25 MΩ and their tip potentials less than 5 mV The electronic arrangement was similar to that used by Edman and Grieve (1964) During impalements the fibres were supported from below by a thin glass rod positioned by a micromanipulator

Solutions All contracture solutions were isotonic to normal Ringer's solution and had a t [K] [Cl] product. Table I gives the composition of all solutions In the following the acture solutions are referred to as 10 K 16 K 20 K, etc Double distilled water was and all chemicals were of analytical grade

Drugs The drugs used in this study were chlorpromazine chloride (Hibernal Leo AB) imipramine h drochloride (Tofranil Geigy) quinidine sulphate (Sigma Chemical Company) and caffeine anhydrous (Sigma Chemical Company)

All experiments were performed at a temperature of 6 0 to 7 5 C

Results

I Drug effects on the time course of the potassium contracture Because of the inhibiting effects of chlorpromazine imipramine and quinidine on the calcium up take in isolated sarcoplasmic reticulum and also on calcium fluxes across the cell membrane (Balzer and Hellenbrecht 1969) these drugs might be expected to affect the time course and the amplitude of the potassium contracture The first series of experiments in the present study was performed to test this point Potassium contractures were produced by rapidly changing from normal Ringer s solution to 117 5 K (Table I) Maximum tension was reached within 1—2 s after the onset of the perfusion with the isotonic potassium solution The tension declined slowly (altogether about 5 per cent) during the subsequent 5—10 s There was then a more rapid decay of tension After relaxation which was completed within 25—35 s the contracture solution was replaced by normal Ringer s this being renewed every 3—5 min Fully reproducible contractures with respect to both total amplitude and time course of the tension development could be produced at inter vals of 15 to 20 min Measurements of the membrane potential showed a complete return to normal (about −90 mV during these periods After 2—4 control contractures chlorpromazine (final concentration 8×10^{-6}—3×10 M) imipramine or quinidine (5×10^{-5}—10^{-4} M [illegible] added to the solutions Fifteen minutes after

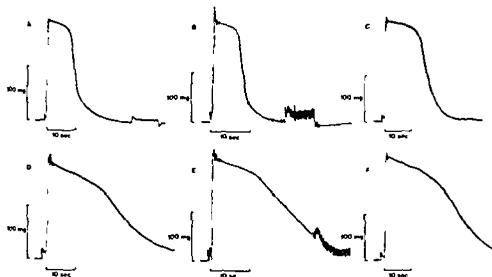


Fig 1 Potassium contractures (117 5 K) in 3 different single fibres A and D B and E C and F respectively Upper row in the absence of drugs (A B and C) Lower row in the presence of 10^{-5} M chlorpromazine (D) 6×10^{-5} M imipramine (E) and 5×10^{-5} M quinidine (F)

the addition of the drugs at least 3—4 reproducible contractures could be obtained at intervals of approximately 20 min At the drug concentrations used characteristic changes of the contractures could be seen The maximum tension output remained completely unchanged (even at the highest drug concentrations) but the relaxation phase was markedly prolonged (Fig 1) The prolongation of the relaxation phase varied to some extent between different fibres but appeared to become more pronounced with the highest drug concentrations used In 14 fibres the increase of the duration of the contracture measured at 50 per cent decay of maximum tension ranged from 50 to 165 (mean 83) per cent

II Drug effects on the relation between peak contracture tension and extracellular potassium concentration It has previously been shown by Hodgkin and Horowicz (1960) that tension development in single muscle fibres is related to log $[K]_0$ by a steep S shaped curve They found that tension starts to develop at 20—25 mM potassium and is almost maximum at 80 mM In the present investigation contracture solutions containing 10 16 20 30 40 80 and 117 5 mM potassium were used All solutions were isotonic with normal Ringer s solution and had a constant [K] [Cl] product which was achieved by using methyl sulphate (see Table I)

Contractures were elicited first by 117 5 K and then at intervals of 15 to 20 min by the other potassium solutions in a random order In each fibre the contracture series was terminated with a contracture in 80 or 117 5 K The experiment was discarded if the fibre produced less than 90 per cent of the contracture tension exhibited in the beginning of the experiment using the same potassium solu

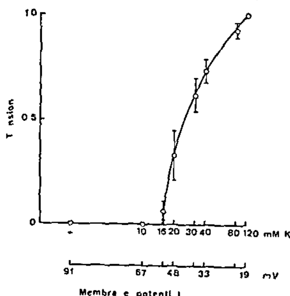


Fig. 2. Relation between peak contracture tension (ordinate), extracellular potassium concentration and membrane potential (abscissa) in seven single fibres. Membrane potential values obtained from bundles of muscles fibres are included.

The membrane potential was measured in some experiments both after the fibre relaxed in the contracture solutions and by a new impalement after re-immersion of the fibre in normal Ringer's solution. Supplementary measurements of membrane potential were also made in bundles of muscle fibres (3—5 fibres in each bundle) immersed in the different contracture solutions. The membrane potential values obtained are included in the data presented in Fig. 2, which summarizes the results obtained in 7 single fibre experiments. As can be seen, tension development started at about 15 mM potassium and was more than 90 per cent of maximum tension at 80 mM. Thus the potassium concentration needed for initiation of tension was lower than in the experiments of Hodgkin and Horowicz (1960). This difference is probably due to the use of methyl sulphate in the present study. As demonstrated by Taylor, Preiser and Sandow (1969) methyl sulphate lowers the mechanical threshold.

After a complete series in Ringer's solution without drugs, the fibre was exposed to solutions containing one of the drugs chlorpromazine (8×10^{-6}—2×10^{-5} M), imipramine or quinidine (5×10^{-5}—10^{-4} M). A series of contractures as described above was carried out in the presence of drugs. As the contractures were very long lasting, especially at the lower potassium concentrations, the fibres were returned to normal Ringer's (with drug added) as soon as tension started to diminish. As Fig. 3 a, b and c show, chlorpromazine, imipramine and quinidine shifted the curve which relates log [K] to peak tension to lower potassium concentrations. Measurements of membrane potential showed that the same degree of depolarization was obtained with the contracture solutions irrespective of the presence or absence of drugs. The results can thus be taken as evidence that chlorpromazine, imipramine and quinidine produce a lowering of the mechanical threshold.

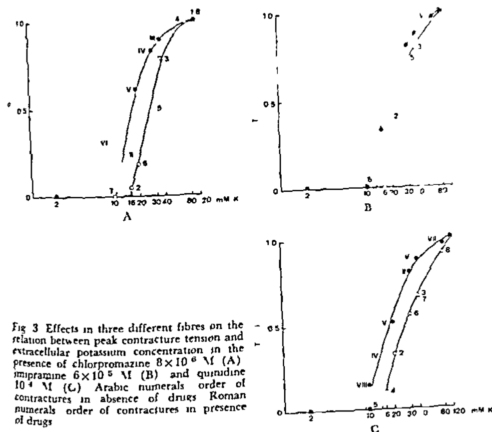


Fig 3 Effects in three different fibres on the relation between peak contracture tension and extracellular potassium concentration in the presence of chlorpromazine 8×10^{-6} M (A) imipramine 6×10^{-5} M (B) and quinidine 10^{-4} M (C) Arabic numerals order of contractures in absence of drugs Roman numerals order of contractures in presence of drugs

TABLE II Contractures induced by qu n dine imipram ne and chlo promaz ne in normal Ring r solution

Experiment no	Drug	Concentration mol l	Tens on per cent of maximum tetan c tension	Lat ncy before tens on secon ls
1	Quin d ne	$3 \times 10^{-}$	51 9	88
2		$3 \times 10^{-}$	4? 5	232
3		$3 \times 10^{-}$	46 9	28
4		$3 \times 10^{-}$	3? 4	126
5		$3 \quad 10^{-}$	45 5	40
6	Im pram ne	$2 \times 10^{-}$	50 0	40
7		$2 \times 10^{-}$	68 3	12
8		$3 \times 10^{-}$	65 0	08
9		$3 \times 10^{-}$	40 1	80
10	Chlorpro na ne	10^{-}	57 1	164
11		10	67 1	30
12		10	64 1	14
13		10^{-}	56 7	32
14		10^{-}	53 2	76
15		10^{-}	60 0	08

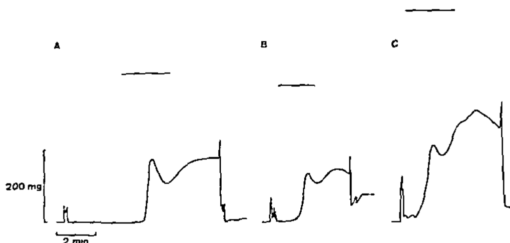


Fig 4 Contractures induced by quinidine (3×10^{-3} M) in three different single fibres A in normal Ringer's B after 14 min in 117 5 K C after 20 min in a calcium free Ringer's containing 10^{-4} M EDTA Horizontal lines tetanic tension

III Contractures induced by chlorpromazine imipramine and quinidine Balzer and Hellenbrecht (1968 1969) demonstrated on frog rectus and sartorius muscles that high concentrations of chlorpromazine ($>5\times10^{-4}$ M) imipramine ($>10^{-3}$ M) and quinidine ($>10^{-3}$ M) produced slowly developing reversible contractures In the present study such contractures were further investigated in single muscle fibres drug concentrations similar to those reported by Balzer and Hellenbrecht (1969) were used

After addition of either of the drugs there was a latency varying from a few seconds to more than 3 min before the contracture started (Table II) The rate of tension development varied from fibre to fibre irrespective of the drug used

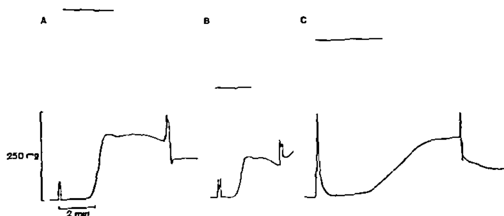


Fig 5 Contractures induced by imipramine (3×10^{-3} M) in three different single fibres A in normal Ringer's B after 18 min in 117 5 K C after 11 min in calcium free Ringer's containing 10^{-4} M EDTA Horizontal lines tetanic tension

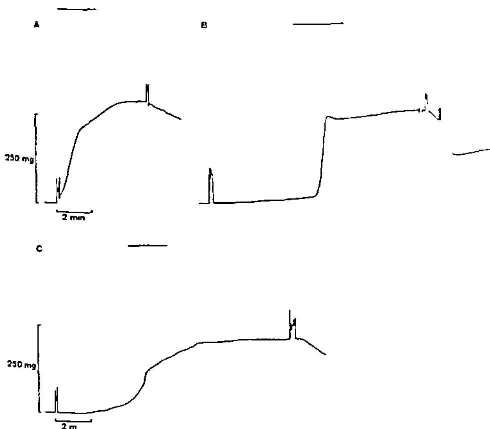


Fig 6 Contractures induced by chlorpromazine (10^{-4} M) in three different single fibres A in normal Ringer's B after 20 min in 117 5 K C after 25 min in calcium free Ringer's containing 10^{-4} M EDTA Horizontal lines tetanic tension

Contractures induced by quinidine and imipramine often had a more rapid tension development than those evoked by chlorpromazine Usually quinidine contractures showed an initial tension peak which was reached within 1—2 min After this tension declined during 30—60 s and then slowly rebuilt to a final level that was sometimes higher than that attained during the initial peak (Fig 4 A) Similar effects could be produced by imipramine (Fig 5 A) and chlorpromazine (Fig 6 A) With the latter drug however tension usually developed more slowly and gradually reached a final maximum within 3—8 min

Microscopic observation revealed that in most cases the fibre was not activated uniformly local contractures could be seen in some parts of the fibre at the same time as other regions were stationary or were being stretched The maximum tension reached during the contractures was about half the tetanus tension (Table II)

The contractures were never completely reversible and local contractures could often be observed when the fibre was returned to normal Ringer's solution

TABLE III Contractures induced by quinidine imipramine and chlorpromazine in 117 5 K

Experiment no	Drug	Concentration mol/l	Tension per cent of maximum tetanic tension	Latency before tension seconds
1	Quinidine	3×10	56 0	3?
2		$3 \times 10^{-}$	39 6	332
3		3×10^{3}	43 2	16
4		3×10^{-5}	44 9	12
5		3×10^{-5}	31 7	44
6		$3 \times 10^{-}$	0	1080
7	Imipramine	3×10^{3}	35 1	36
8		3×10^{-4}	32 6	640
9		3×10^{-5}	33 8	112
10	Chlorpromazine	10^{-4}	54 3	10
11		10^{4}	48 6	04
12		10^{-4}	14)	440

irreversibility was particularly conspicuous *after the second contracture* phase had developed At this stage the fibres generally exhibited a granular and fragmented appearance

In a few fibres a comparison was made between caffeine contractures and those produced by chlorpromazine imipramine and quinidine It was found in confirmation of the results described by Luttgau and Oetliker (1968) that caffeine (4—5 mM) produced a contracture that started immediately after exposure to the drug *reached a maximum within 5—10 s* and had an amplitude of about 90 per cent of the tetanic tension The contractures produced by chlorpromazine imipramine and quinidine as described above showed a much slower tension development and in no case was the same high tension attained as in a caffeine contracture

It is well known that the caffeine contracture develops without any substantial change of the membrane potential (Axelsson and Thesleff 1958) As the contractures elicited by chlorpromazine imipramine and quinidine developed very slowly it was possible to make measurements of the membrane potential during the course of tension development In 3 fibres where contractures were induced by 10^{4} chlorpromazine the membrane potential fell by 3—8 mV during the initial phase of the contracture When maximum was *reached the membrane potential continued to fall* At this stage the contracture effect could not be reversed when the fibres were returned to normal Ringer s solution

Caffeine contractures can be produced in a depolarized muscle and also in a calcium free medium (Axelsson and Thesleff 1958) It was therefore considered of interest to find out whether chlorpromazine imipramine and quinidine could induce contractures under these conditions

In 5 single fibres immersed for 10—20 min in 117 5 K (membrane potentials —18 to —20 mV) it was found that contractures could be elicited by the same concentrations of quinidine as in normal Ringer s (Table III Fig 4 B) However in one

TABLE IV Contractures induced by quinidine imipramine and chl rpron z in c Ringer s containing 10^{-4} M EDTA

Experiment no	Drug	Concentration	Tension per cent of maximum tetanic tension	Latency bet t n r cor
1	Quinidine	3×10	(9	03
2		3×10	3/ 5	03
3		3×10	(4 0	03
4		3×10^{-3}	(4 1	03
5		3×10⁻	59 7	2
6		3×10^{-3}	49 /	3
7		3×10	7 9	1
8	Imipramine	2×10	/3 3	0
9		2×10	54 9	04
10		3×10^{-3}	55 0	04
11		3 10	71 0	80
12	Chlorpromazine	1×10^{-4}	43 2	89
13		1×10^{-4}	67 9	60
14		2×10^{-4}	19 7	24

fibre exposed to 3×10^{-3} M quinidine in 117 5 K for 18 min no contracture response was obtained Also chlorpromazine and imipramine evoked contractures in depolarized fibres The amplitude of these contractures however was somewhat lower than observed in normal Ringer s solution (Table III Fig 5 B and Fig 6 B)

Fibres soaked for 10—30 min in a calcium free Ringer s solution with 10^{-4} M EDTA responded to the same concentrations of quinidine as did normal fibre (Table IV and Fig 4 C) The amplitude of the contractures was about the same as in normal Ringer s

Similar to quinidine chlorpromazine and imipramine could elicit contractures in a calcium free solution (Table IV Fig 5 C and Fig 6 C) In 3 out of 4 fibres 3×10^{-3} M imipramine in calcium free Ringer s induced a transient tension development after which the fibre was almost completely relaxed This was succeeded by a second development of tension with irreversible changes in the fibres (Fig 5 C)

Discussion

Effects on the mechanical threshold and on the potassium contracture The steep S-shaped curve relating peak tension to the concentration of extracellular potassium (Hodgkin and Horowicz 1960) is shifted to lower potassium concentrations in the presence of lyotropic anions (Isaacson and Sandow 1967) caffeine (Etzensperger and Gascolli 1963 Sandow *et al* 1964 Luttgau and Oetliker 1968 Taylor Preiser and Sandow 1969) and quinine (Benoit Carpeni and Przybyslawski 1961 Sandow *et al* 1964) A shift to higher potassium concentrations is produced by local anesthetics such as tetracaine cocaine and procaine (Luttgau and Oetliker 1968 Etzensperger 1970 a) lanthanum (Andersson and Edman 1972) and by an increase in the extracellular calcium concentration (Luttgau 1963 Frankel

and Lannergren 1966 Etzensperger 1970 b) The results of the present study have clearly shown that in the presence of chlorpromazine, imipramine and quinidine the curve is shifted to lower potassium concentrations As these drugs do not affect the relation between extracellular potassium concentration and membrane potential it can be concluded that there is a true lowering of the mechanical threshold

The effects on the mechanical threshold and on the time course of the potassium contracture produced by chlorpromazine imipramine and quinidine may be due to actions on the cell membrane on intracellular structures, or on both Similar to local anesthetics these drugs are membrane stabilizers (Shanes 1958 a b, van Zwieten 1969 Langslet 1970 Langslet *et al* 1971) and thus inhibit ion fluxes across cell membranes It might be expected that these actions would be of relevance to their effects on tension production During depolarization of a muscle by high external potassium concentrations the influx of calcium is markedly increased (Bianchi and Shanes 1959 Weiss and Bianchi 1965) This calcium influx does not directly initiate contractile activity but there are reasons to believe that it may play a role as a trigger of calcium release from intracellular stores (Endo Tanaka and Ogawa 1970 Ford and Podolsky 1970) Interference with this calcium trigger mechanism may be thought to influence the production of tension Local anesthetics such as tetracaine and procaine reduce the maximum contracture tension and shift *the curve which relates peak tension to extracellular potassium concentration to* [illegible]er potassium values (Luttgau and Oetliker 1968 Etzensperger 1970 a), and it [illegible] indeed been suggested that these actions are due to a membrane stabilizing effect (Etzensperger 1970 a) It may thus be that the local anesthetics by their membrane stabilizing action reduce the inflow of calcium from the extracellular medium and thereby diminish the release of activator calcium inside the cell Such a mechanism however cannot explain the actions of chlorpromazine imipramine and quinidine as elucidated in the present study These agents, contrary to conventional local anesthetics do not affect the maximum contracture tension and they lower the mechanical threshold These findings strongly suggest that their membrane stabilizing actions are of less importance for tension production than are their effects on intracellular structures

The lowering of the mechanical threshold and the shift to the left of the curve relating peak tension to extracellular potassium concentration produced by chlorpromazine imipramine and quinidine may be explained by effects on intracellular calcium stores Assuming that tension is quantitatively related to the free myoplasmic calcium concentration (which determines the amount of calcium bound to the active sites), the effectiveness of a given depolarization in releasing calcium from the sarcoplasmic reticulum might be increased in the presence of these drugs and result in an increased tension production (Isaacson and Sandow 1967) Another possibility worthy of consideration is that a rise of the free myoplasmic calcium concentration at rest (still subthreshold) is produced by drug mediated release of calcium from the sarcoplasmic reticulum (Bondani and Karler 1966, Carvalho 1968) The amount of calcium released upon depolarization of the membrane would add to the relatively

high resting calcium concentration and thereby produce a potenti d ten n put

The prolongation of the relaxation phase of the po ss ium contracture proc by the drugs studied perhaps cannot be entirely explained by the effects mentio above but suggests that there is also a slowing of the calcium uptake in t sarcoplasmic reticulum In support of such a mechanism it ha been de non trated that chlorpromazine imipramine and quinidine all inhibit the calcium pump in isolated reticulum (Balzer Makino e and Hasselbach 1968

Contracture effects of chlorproma ine imipramine and quinidine It is n that high concentrations of quinidine ($> 10^{-3}$ M) and its optical isomer quinine can produce contractures in skeletal muscle (Benoit Carpeni and Fr   1964 Isaacson and Sandow 1967 Isaacson Yamaji and Sandow 1970 Huddar 1971 a b) Contractures can also be produced by chlorpromazine and imipramine (Balzer and Hellenbrecht 1968 1969) The mechanisms of these contractures have been studied but are still not fully understood Balzer and Hellenbrecht (1969) found that a contracture producing concentration of chlorpromazine (10^{-4} M) increased the ratio of calcium influx to calcium efflux by 11 per cent Chlorpromazine in this concentration had previously been shown to produce a 70 per cent inhibition of calcium uptake in isolated sarcoplasmic reticulum (Balzer Makinose and Hasselbach 1968) On the basis of these findings it was presumed (Balzer and Hellenbrecht 1969) that the intracellular concentration of free calcium was raised sufficiently to cau e activation of the contractile system However an increased calcium influx would not seem to be essential for the development of contracture as chlorpromazine imipramine and quinidine have been shown in this study to induce contractures in the ab ence of extracellular calcium The e findings strongly suggest that contracture is produced by release of calcium from a cellular store The fact that quinidine and quinine have been found to release calcium from isolated sarcoplasmic reticulum (Bondani and Karler 1966 Carvalho 1968) supports this idea

An interesting finding in the present study was that the caffeine contracture developed much more rapidly than the contractures produced by chlorpromazine imipramine and quinidine This may be due to differences in the ability to permeate the cell membrane as was pointed out by Huddart (1971 a) Caffeine is a weak base with a pK_a of 0 8 (Bianchi 1968) This means that at pH 7 0 all of the caffeine exists in its uncharged form and may easily penetrate the cell membrane The pK_a for chlorpromazine is 9 30 for imipramine 9 5 (Green 1967) and for quinidine 8 8 As these bases are evidently almost completely protonated at pH 7 0 they may be presumed to pass into the cell much more slowly than does caffeine In accor dance with this view it has been shown that both the rate of tension development and the total amplitude of the contracture produced by quinine are potentiated by rais ing the pH and hence increasing the portion of unchanged quinine (Isaacson Yamaji and Sandow 1970 Huddart 1971 b) Another possibility is that the differ ence in the time course of the contractures may reflect differences in the site of

action between caffeine on the one hand, and chlorpromazine imipramine and quinidine on the other Studies on isolated sarcoplasmic reticulum (Carvalho 1968, Bondani and Karler 1970) suggest that caffeine might act on a different calcium fraction than does quinine and quinidine The experiments of Benoit, Carpeni and Przybyslawski (1964) can be taken as further support for this presumption These authors found that at a time when caffeine contractures could no longer be obtained after frequent application of caffeine, the quinine contracture could still be elicited Furthermore, whilst the caffeine contracture is blocked by procaine the quinine contracture is not affected (Isaacson Yamaji and Sandow 1970)

The membrane potential is not affected by caffeine, and contractures can be evoked also in a depolarized muscle (Axelsson and Thesleff 1958) Previous studies on quinine contractures in depolarized muscle have produced conflicting results Benoit Carpeni and Przybyslawski (1964) found that quinine did not induce contractures in depolarized frog muscle and Huddart (1971 a) reported the same finding in crab muscle fibres Isaacson and Sandow (1967) on the other hand found that quinine did produce contractures in depolarized frog sartorius which reached a tension of about 40 per cent of that produced during a potassium contracture The cause of this discrepancy in results is unclear The present experiments have demonstrated however that contractures can be elicited by chlorpromazine imipramine and quinidine in depolarized single muscle fibres of the frog It was shown moreover that during the initiation of the contractures there was only a slight fall in membrane potential Taken together these findings probably mean that the contractures are not mediated by way of depolarization of the cell membrane The contractures are most likely due to a direct effect of the drugs upon some intracellular calcium store that leads to a release of calcium into the myofibrillar space

I wish to thank Professor K A P Edman for his interest during the course of this investigation and for many helpful discussions The skilful technical assistance of Mrs Britta Kronborg is gratefully acknowledged

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The Effects of Vasoconstrictor Fibre Stimulation on the Consecutive Vascular Sections of the Small Intestine of the Cat during Prolonged Regional Hypotension

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Abstract

HAGLUND U and O LUNDGREN *The effects of vasoconstrictor fibre stimulation on the consecutive vascular sections of the small intestine of the cat during prolonged regional hypotension* Acta physiol scand 1972 85 547—558

A plethysmographic technique was used on the cat to investigate the effects of electrical stimulation of the regional sympathetic vasoconstrictor fibres on intestinal consecutive vascular sections during prolonged hypotension at approximately 55 or 30 mm Hg produced by graded arterial occlusion The initial peak resistance and the capacitance responses declined continuously throughout the hypotensive period the rate of decline being higher the lower the perfusion pressure After 2 5 h hypotension at 30 mm Hg the nervous vasoconstrictor effects on the resistance and capacitance vessel amounted to 50 and 30 per cent of control respectively The reactivity of the precapillary sphincters as reflected in the capillary filtration coefficient (CFC) was largely unaltered during the hypotension at the 55 mm Hg level At the lower perfusion pressure a marked increase of CFC was noted between and during nervous stimulations Mean capillary hydrostatic pressure was not significantly changed by nervous activation but seemed to increase in the latter half of most experiments at 30 mm Hg When releasing the arterial clamp after a 30 mm Hg hypotension the whole cardiovascular system seemed to derange progressively Mucosal ulcerations and bleedings were regularly found in all these animals

It has been repeatedly claimed that the splanchnic circulation is of great importance in the development of shock (see Texter 1963) Thus pooling of blood in the splanchnic vessels has been proposed to be of functional importance in the shocked organism (Wiggers *et al* 1946 Texter 1963) Further Lillehei (1957) showed on dogs that preventing intestinal ischemia during periods of hemorrhagic shock by means of cross circulation reduced the mortality rate from 90 to 10 per cent Recently the possible release of depressor factors from the splanchnic area during ischemia has been much discussed (see *e g* Lefer 1970 Selkurt 1970 Haglund and Lundgren 1972 b)

In this laboratory the vascular reactions in cat skeletal muscle have been studied during regional hypotension and during hemorrhage with a plethysmographic technique (Lewis and Mellander 1962 Mellander and Lewis 1963 Lundgren Lundvall and Mellander 1964) The present investigation represents one part in a series of studies of the cat intestinal vessels during regional hypotension and hemorrhagic shock performed in a manner similar to that of Mellander *et al* The effects of the sympathetic vasoconstrictor fibres on the intestinal vessels were in the present study investigated during prolonged regional hypotension at two pressure levels utilizing a plethysmographic technique (Folkow *et al* 1963) that allows a quantitative analysis of the reactions within the resistance exchange and capacitance vessels In an earlier paper (Haglund and Lundgren 1972 b) results were reported from experiments in which the reactions of the e series coupled vascular sections were studied in the denervated intestine when the regional perfusion pressure was lowered Thus it was possible to compare the present results with that study as well as with the works of Mellander *et al* Parts of this study have been published in a preliminary form (Haglund and Lundgren 1972 a)

Methods

Operative procedures and determination of blood flow Experiments were performed on 2[illegible] weighing 2—5 kg anesthetized intravenously with chloralose (50—65 mg/kg b w) after induction with ether The cats had been deprived of food for 12 h and had no obvious signs of intestinal infection Immediately after anesthesia an intravenous infusion of a 10 % glucose solution containing 10 meq $NaHCO_3$ per 100 ml was started at a rate of 0 1 ml/min and continued throughout the experiment

The details of the operative technique used were presented in previous publications (Folkow *et al* 1963 Haglund and Lundgren 1972 b) and the method is below only summarized An isolated jejunal segment of 30—40 g was chosen for the experiment and the remainder of the intestinal tract was extirpated An adjustable clamp was placed around the superior mesenteric artery so that the arterial inflow pressure to the intestinal vascular bed could be set at any desired level The mesenteric vein draining the jejunal segment and its lymph nodes was cannulated and connected to a drop recorder operating an ordinate writer Venous outflow pressure was set at approximately 10 mm Hg (13—15 cm H_2O) and continuously checked by a water manometer in order to minimize the passive elastic recoil of the capacitance vessels during sympathetic vasoconstrictor fibre activation (Folkow *et al* 1964 a Öberg 1967) Arterial pressure was recorded by means of 2 mercury manometers from the left femoral artery and from a major branch of the superior mesenteric artery placed below the screw clamp Arterial blood samples for pH and P_{CO} measurements could be taken *via* a cannula in the right femoral artery

The splanchnic nerves were cut bilaterally and their distal ends placed in ring electrodes for stimulation (4 or 8 imp/s 6 ms 6 V) with a Grass stimulator The adrenals were tied off and 10 mg cortisone acetate (Upjohn) was given i m The vagal fibres to the small intestine were left intact

In 3 expts where the intestine was perfused at a pressure of 30 mm Hg hind limb blood flow was also measured by means of a drop recorder connected to the femoral vein The paw was tied off by ligatures so that the recorder measured mainly muscle blood flow and the limb was denervated by cutting the femoral and sciatic nerves

B Plethysmographic technique and determination of the capillary filtration coefficient To allow a continuous recording of changes in tissue volume the jejunal segment and its lymph nodes were enclosed in a perspex plethysmograph filled with Tyrode s solution (For details see Haglund and Lundgren 1972 b where also the determination of the capillary filtration coefficient (CFC) is discussed) As mentioned earlier venous outflow pressure was set at a higher level (10—11 mm Hg) than in the preceding study to minimize passive elastic recoil of the capacitance vessels It therefore seemed justified to assume a higher mean capillary pressure

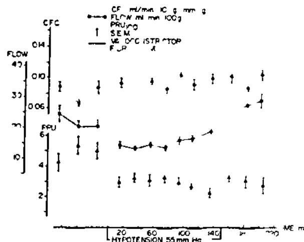


Fig 1 The effects of a prolonged local hypotension (arterial inflow pressure approximately 55 mm Hg) on blood flow regional flow resistance and capillary filtration coefficient (CFC) of the small intestine The regional sympathetic vasoconstrictor fibres were intermittently stimulated as indicated by bars on the abscissa Note that the flow and resistance values during nervous vasoconstriction depicted in the Fig were registered during the steady state phase of sympathetic vasoconstriction (n = 10)

($\bar{P}$) than the earlier assumed 15 mm Hg In the calculations of CFC $\bar{P}$ has therefore been set to 20 mm Hg The error in the CFC determination introduced by assuming a certain $\bar{P}$ is earlier discussed at length (Folkow *et al* 1963 Haglund and Lundgren 1972 b)

C The acid base balance of the experimental animal Arterial blood pH and P_{CO_2} and pH of the venous outflow from the intestine were in most experiments repeatedly checked with an Astrup pH meter (pH meter 27 with a microelectrode unit)

D Experimental procedures After enclosing the intestinal segment in the plethysmograph the cat was allowed to "rest" for approximately 30 min in order to reach a control steady state in arterial pressure intestinal blood flow and tissue volume The sympathetic vasoconstrictor fibres were then activated for 20 min and after a new poststimulatory control period the arterial inflow pressure to the intestine was reduced to 55 or 30 mm Hg for about 150 min During this period of hypotension the vasoconstrictor fibres were intermittently stimulated during three 20 min periods and during a subsequent post hypotension period of 60 min another vasoconstrictor fibre activation was performed To minimize intravascular cell aggregation 1 ml of low molecular dextran was given to the animal every 20 min throughout the experiment

Results

A Control experiments

In 3 control experiments arterial inflow pressure to the small intestine was left undisturbed while the sympathetic fibres were intermittently stimulated as during hypotension After 4 h the peak resistance response was if anything somewhat larger than at the beginning of the control period The capacitance response amounted to approximately 95 per cent of control

B Prolonged hypotension at 55 mm Hg

1 Resistance vessels Fig 1 illustrates the reactions within the consecutive vascular sections of the small intestine before during and after a local hypotension at about 55 mm Hg Throughout the experiments the sympathetic vasoconstrictor fibres were intermittently stimulated at 4 or 8 imp/s as indicated by a black bar on the abscissa

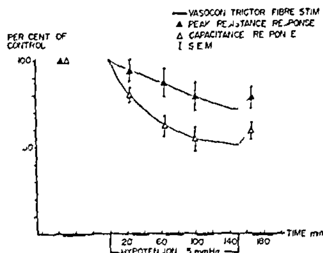


Fig 2 The effects of regional vasoconstrictor fibre activation on the intestinal resistance (peak resistance response) and capacitance vessels during and after a prolonged regional reduction of arterial inflow pressure to about 25 mm Hg The vascular responses are expressed in per cent of control values (n = 10)

It should be pointed out that the results illustrated in Fig 1 for blood flow and resistance were recorded during the '*steady state*' phase of nervous vasoconstriction (Folkow *et al* 1964 a) The sequence of vascular responses were quantitatively similar whether the nerves were stimulated at 4 or 8 imp/s and, hence the results from the 2 series of experiments are reported together The control values illustrated in Fig 1 are within the 'normal' range reported earlier (Lundgren 1967 Haglund and Lundgren 1972 b)

As shown in Fig 1 vasoconstrictor fibre stimulation produced a slight increase in the 'steady state' resistance during the control period Upon lowering arterial inflow pressure to 25 mm Hg resistance was rapidly reduced from 4—5 to 3 PRU$_{100}$ units and a superimposed vasoconstrictor fibre stimulation produced an approximately 10 per cent increase of resistance in the early phase of hypotension In late phases however blood flow or resistance were hardly at all affected by the stimulations as compared with the immediate prestimulatory values As reported earlier (Haglund and Lundgren 1972 b) intestinal blood flow tended to increase after about 80 min of hypotension When releasing the partial arterial occlusion resistance remained reduced below the control values Furthermore resistance did not increase upon activation of the vasoconstrictor fibres and resistance tended to decrease gradually throughout the post hypotensive period

During vasoconstrictor fibre stimulation the intestinal resistance vessels respond in a typical manner (Folkow *et al* 1964 a) characterized by an initial peak constrictor response within 1 min followed by a gradual flow increase that reaches a new 'steady state' level of moderately raised resistance within 2—4 min The effect of local hypotension on the peak response is shown in Fig 2 It can be seen that the peak resistance response expressed in per cent of control gradually declined during the hypotensive period

2 Exchange vessels In the control period preced... the hyp... a marked reduction of CFC during vasoconstrictor ... stimulation ... Folkow *et al* 1964 b) but during the hypotensive period this CFC ... became ... less pronounced and it remained small also during th... posthypotensive ...

3 Mean capillary hydrostatic pressure No change o... capillary hydrostatic pressure was observed during hypotension to judge by ... tissue volume observed between stimulations or during the steady state ... vasoconstriction When releasing the partial arterial occlusion ... increase of tissue volume was occasionally seen corresponding to a rise in capillary pressure

4 Capacitance vessels When the sympathetic vasoconstrictor fibre... in the control period an initial rapid decrease of tissue volume was registered ... which tissue volume remained constant This reduction in tissue volume ... mainly due to a decrease of the blood content of the capacitance vessels as previously demonstrated (Mellander 1960 Wallentin 1966) and it amounted to 2 7 ± 0 3 ml/100 g (mean ± S E n = 10 see also Folkow *et al* 1964 a) It should be pointed out that venous outflow pressure was in those experiments kept at such a level as to minimize passive elastic recoil of the capacitance vessel (see Methods section A) Fig 2 shows that the neurogenic capacitance response was markedly reduced during the hypotensive period reaching a value of about 50 per cent of control at the end of the hypotension and it never returned to control in the posthypotensive period

5 Posthypotensive condition of the experimental animals The experimental animals seem to tolerate a 2 5 h period of intestinal hypotension at 55 mm Hg rather well A slight but continuous fall in systemic arterial blood pressure was observed after restoring the intestinal perfusion pressure but no animal died during the posthypotensive period No bleedings were found in the intestinal mucosa at a post mortem examination

C Prolonged hypotension at 30 mm Hg

1 Resistance vessels Fig 3 illustrates the reactions within the consecutive vascular sections of the small intestine before during and after a 2 h period of local hypotension at approximately 30 mm Hg The sympathetic vasoconstrictor fibres were intermittently stimulated (black bars on abscissa) throughout the experiments Again the results obtained with 4 or 8 imp/s were similar and are reported together

The changes observed during a 30 mm Hg hypotension were as regards the resistance vessels qualitatively similar to those described above during hypotension at the 55 mm Hg level (Fig 1) Quantitatively however they differed in so far as the intestinal blood flow started to gradually increase at an earlier stage and posthypotensive "reactive hyperemia was much more pronounced

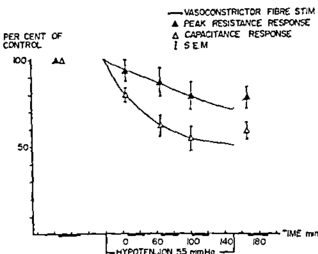


Fig 2 The effects of regional vasoconstrictor fibre activation on the intestinal resistance (peak resistance response) and capacitance vessels during and after a prolonged regional reduction of arterial inflow pressure to about 55 mm Hg The vascular responses are expressed in per cent of control values (n = 10)

It should be pointed out that the results illustrated in Fig 1 for blood flow and resistance were recorded during the *steady state* phase of nervous vasoconstriction (Folkow *et al* 1964 a) The sequence of vascular responses were quantitatively similar whether the nerves were stimulated at 4 or 8 imp/s and hence the results from the 2 series of experiments are reported together The control values illustrated in Fig 1 are within the normal range reported earlier (Lundgren 1967, Haglund and Lundgren 1972 b)

As shown in Fig 1 vasoconstrictor fibre stimulation produced a slight increase in the steady state resistance during the control period Upon lowering arterial inflow pressure to 55 mm Hg resistance was rapidly reduced from 4—5 to 3 PRU_{100} units and a superimposed vasoconstrictor fibre stimulation produced an approximately 10 per cent increase of resistance in the early phase of hypotension In late phases however blood flow or resistance were hardly at all affected by the stimulations as compared with the immediate prestimulatory values As reported earlier (Haglund and Lundgren 1972 b) intestinal blood flow tended to increase after about 80 min of hypotension When releasing the partial arterial occlusion resistance remained reduced below the control values Furthermore resistance did not increase upon activation of the vasoconstrictor fibres and resistance tended to decrease gradually throughout the post hypotensive period

During vasoconstrictor fibre stimulation the intestinal resistance vessels respond in a typical manner (Folkow *et al* 1964 a) characterized by an initial peak constrictor response within 1 min followed by a gradual flow increase that reaches a new steady state level of moderately raised resistance within 2—4 min The effect of local hypotension on the peak response is shown in Fig 2 It can be seen that the peak resistance response expressed in per cent of control gradually declined during the hypotensive period

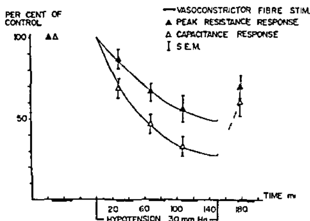


Fig 4 The effects of regional vasoconstrictor fibre activation on the intestinal resistance (peak resistance response) and capacitance vessels during and after a prolonged regional reduction of arterial inflow pressure to about 30 mm Hg The vascular responses are expressed in per cent of control values (n = 11)

5 Posthypotensive condition of the experimental animals In the posthypotensive period the experimental animals seemed to derange progressively with a marked rapid fall in arterial blood pressure (from 90 to 30 mm Hg in 30 min) 2 out of 11 animals died within one hour after the hypotensive period Macroscopic post mortem examination of the small intestine showed bleedings in the mucosa and microscopic examinations revealed capillaries filled by erythrocytes as well as mucosal edema and bleedings

D Skeletal muscle blood flow

In three experiments blood flow from the denervated hind limb muscles was measured concomitantly to the intestinal blood flow recording During intestinal hypotension at 30 mm Hg muscle blood flow remained unchanged but when arterial pressure decreased progressively during the posthypotensive period muscle blood flow fell correspondingly *i e* muscle flow resistance remained largely the same throughout the experiment

E Blood pH and P_{CO_2} determinations

Arterial blood pH and P_{CO_2} as well as intestinal venous blood pH were repeatedly determined during most experiments In the control period arterial P_{CO_2} measured 28 ± 2 mm Hg (mean $\pm$ S E n = 14) falling to 26 ± 1 (n = 9) during the hypotension and in the posthypotensive period Similar results were obtained at the two different hypotensive levels

The measured values in arterial and intestinal venous pH are summarized in Table I showing that arterial pH remained unchanged throughout the hypotensive periods probably a result of the slow intravenous infusion of a bicarbonate solution (see Methods section C) Intestinal venous pH on the other hand was mark decreased during low pressure perfusions

TABLE I Values of arterial and intestinal venous blood pH before during and after regional arterial hypotension at 55 and 30 mm Hg Mean values ± S E n = number of observation

		before hypotension	hypotension		after hypotension
			early half	late half	
Hypotension 30 mm Hg	Arterial	7 38±0 04 n = 8	7 35±0 02 n = 8	7 36±0 02 n = 8	7 39±0 02 n = 8
	Intestinal venous	7 23±0 03 n = 6	7 10±0 04 n = 5	7 06±0 04 n = 6	7 15±0 03 n = 6
Hypotension 55 mm Hg	Arterial	7 36±0 06 n = 5	7 38±0 06 n = 3	7 31±0 03 n = 3	7 33±0 02 n = 5
	Intestinal venous	7 25±0 02 n = 4	7 17±0 05 n = 3	7 11±0 02 n = 3	7 19±0 04 n = 5

Discussion

Stimulation of the regional sympathetic vasoconstrictor fibres to the small intestine results in an initial marked increase of vascular resistance as a result of a constriction of the resistance vessels (peak resistance response) Concomitantly the capacitance vessels constrict as indicated by an abrupt decrease of tissue volume Within [illegible] min after the onset of the constrictor fibre stimulation intestinal blood flow [illegible] increases reaching a new steady state level slightly below control while the [illegible]rogenic capacitance response remains largely unaltered throughout the stimulation period (at least when venous outflow pressure is kept above 10 cm H O to avoid venous collapse Folkow *et al* 1964 a) During the steady state phase of vasoconstriction flow resistance seldom increases more than 100 per cent above control even at the highest physiological stimulation rates, while the capillary filtration coefficient (CFC) is more drastically reduced often to values below half the control (Folkow *et al* 1964 b) This vascular response pattern may reflect a restribution of blood flow within the intestinal wall (Folkow *et al* 1964 b)

The intention of the present study was to investigate how the above mentioned neurogenic intestinal vascular adjustments were altered during and after prolonged local hypotension The latter procedure presumably induces an accumulation of metabolites which in turn were probably responsible for the decreased responsiveness of the consecutive vascular sections (see below) However alternative less plausible explanations exist as discussed at some length by Lewis and Mellander (1962) Suffice to say here that trauma to the isolated nerves could account for at most a 5 per cent decrease of nervous responsiveness as judged by control experiments

The abovementioned term accumulation of metabolites designates a change of the chemical environment of the vascular smooth muscles including the deficiency of some nutrient(s) a change that cause a vasodilatation Thus during sympathetic stimulation a balance is created between the constricting effects of the neurotransmitter and the vasodilator effects of the accumulated metabolites In the present

study it was demonstrated that the impact of the metabolites becomes gradually more dominant the longer and more severe the hypotension is as judged by the deteriorating neurogenic vascular responses (Fig 2 and 4) A decreased vascular responsiveness was also recorded in the posthypotensive period It appears as if the deterioration is more pronounced for the capacitance than for the resistance vessels in the intestine at least when the neurogenic effect on the resistance vessels was estimated from the initial peak vasoconstriction The opposite finding was reported by Lewis and Mellander (1962) for skeletal muscle When muscle blood flow was reduced to half of the control the neurogenic capacitance response was maintained at the control level for at least 20 min while the resistance respon e was rapidly reduced to 25 per cent of the control These observations on skeletal muscle should be compared with those reported in this study on the intestine at the 30 mm Hg hypotension (Fig 3 and 4) where regional blood flow amounted to approximately half the control value during the hypotension

The present technique does not only allow an analysis of the neurogenic effects on the intestinal resistance and capacitance function but al o on the capillary filtration coefficient (CFC) CFC is a measure of the water conductivity of the capillary endothelium and is dependent on the size and number of pores per capillary unit area as well as on the capillary surface area available for exchange The latter factor in turn is dependent on the tone of the so-called precapillary sphincters which determines the number of capillaries open to flow The reduction of intestinal CFC seen during sympathetic stimulation reflected in all probability a closure of such precapillary sphincters (Folkow *et al* 1964 b) This reduction of intestinal CFC became less pronounced during hypotension and to a certain extent also in the posthypotensive period probably due to a deteriorated local chemical environment This effect was particularly pronounced at the lower perfusion level during which CFC very seldom reached control values even during neurogenic vasoconstriction It is not possible with the present technique to elucidate how the neurogenic regional redistribution of blood flow (Folkow *et al* 1964 b) may be changed during hypotension

The vascular reactivity to nervous stimulation was declining more rapidly for the intestinal capacitance vessels than for the resistance ones (Fig 2 and 4) As noted above the opposite finding was reported for skeletal muscle by Lewis and Mellander (1962) These authors ascribed their observation to a quantitative difference in sensitivity to metabolites of the various consecutive vascular sections Such a conclusion implies that the series coupled vascular sections are influenced by similar concentrations of metabolites an assumption which may hold true in the fairly homogenous skeletal muscle vascular bed As regards the intestinal vascular bed however one certainly cannot make any corresponding assumption since metabolic rate differs markedly in the various layers of the gut It seems for example reasonable to assume that metabolism proceeds at a considerably higher rate at the bases of the crypts where cell regeneration takes place than *e g* in the muscularis This for example i indicated by the greatly different rates of blood flow in these tissue

compartments (Lundgren 1967) In case the main part of the capacitance function is anatomically located in a different intestinal wall layer than the resistance function it is clear from the discussion above that the implicit assumption of Lewis and Mellander may not hold true for the intestine Consequently it is not possible to explain with any certainty why the nervous responsiveness of the intestinal capacitance vessels declines at a faster rate than that of the resistance vessels

Mean capillary hydrostatic pressure in the intestinal vascular bed remains unchanged during neurogenic vasoconstriction (Folkow *et al* 1964 a) while a capillary pressure reduction and hence an absorption of fluid occurs in skeletal muscle in the same situation (Mellander 1960) Throughout the present study no change of capillary pressure was observed during sympathetic stimulation as compared to prestimulatory control Thus the neurogenic constriction of precapillary and postcapillary resistance vessels must be so well balanced even during prolonged hypotension as to keep mean capillary pressure largely unchanged This conclusion is also supported by the concomitant decline of vascular reactivity in both resistance (mainly precapillary located) and capacitance vessels (mainly postcapillary located Fig 2 and 4) In the studies of the skeletal muscle vessels (Lewis and Mellander 1962) a completely different vascular response pattern was observed Since the neurogenic vasoconstriction was here better maintained for the postcapillary capacitance vessels mean capillary pressure could ultimately increase above control in connection with sympathetic vasoconstriction inducing a transcapillary filtration It was proposed by Lewis and Mellander (1962) that such a loss of fluid from the vascular system may contribute to the deterioration seen in severe shock

From this discussion one may conclude that the intestinal and skeletal muscle vascular beds behave quite differently as to their responses to sympathetic vasoconstrictor fibre stimulation in situations of prolonged hypotension The interesting pathophysiological mechanism in severe shock proposed for skeletal muscle and referred to above (Lewis and Mellander 1962) seems to be of insignificant relevance with respect to the intestinal vascular bed

Other mechanisms may however here be of greater importance Firstly one may infer a certain pooling of blood in the intestine during and after hypotension (Wiggers *et al* 1946 Texter 1963) as judged by the declining nervous responsiveness of the capacitance vessels (Fig 2 and 4) After approximately 2 h hypotension at 30 mm Hg the capacitance response averaged only about 30 per cent of control If it is assumed that approximately 400 ml of blood are expelled from the splanchnic area of man during maximal nervous vasoconstriction (*cf* Folkow and Neil 1971) the results of the present study suggest that some 300 ml may become pooled during prolonged hypotension due to the decreased nervous responsiveness alone

Secondly a steady slow increase of tissue volume was observed in the latter half of a 30 mm Hg hypotension period both between and during stimulations and it continued also after the release of the arterial obstruction A similar train of events was seen in the previous series of experiments at the corresponding perfusion pressure level (Haglund and Lundgren 1972 b) These augmentations of tissue volume

may reflect a transcapillary filtration due to an increased mean capillary pressure caused by the derangement of the autoregulatory properties of the intestinal resistance vessels It should be underlined that even a small increase of mean capillary pressure in the small intestine may induce a large loss of fluid from the vascular system since the water conductivity of the intestinal capillaries is comparatively large

Thirdly it was noticed in the present and in the preceding series of experiment that upon releasing the partial arterial occlusion after a 30 mm Hg hypotension the whole cardiovascular system seemed to derange progressively A rapidly falling systemic arterial blood pressure was evident within minutes after releasing the arterial clamp Furthermore in this study 2 out of 11 animals died within 1 h after the hypotension and intestinal mucosal bleedings and even ulcerations were frequent at the post mortem examination Such observations were made following a 30 mm Hg hypotensive period when intermittent vasoconstrictor fibre stimulation were performed but *not* in the previous series of experiments where no such stimulation were involved (Haglund and Lundgren 1972 b) As indicated in the introduction and discussed at some length in the previous publication these observations possibly indicate that some substance(s) are released from the ischemic intestine into the general circulation (*cf* Lillehei 1957 Lefer 1970) These factor(s) seem unable to change skeletal muscle flow resistance which was unaltered after the hypotensive period in the face of slowly decreasing intestinal flow resistance

Results were presented in an earlier publication suggesting that the autoregulation of the denervated intestinal vascular bed seen during prolongd local hypotension is mainly of myogenic nature in the *early* part of low pressure perfusion (Haglund and Lundgren 1972 b) However in the course of 2—3 h of a 30 mm Hg hypotension accumulating metabolites no doubt become more and more important in determining the tone of the intestinal vascular smooth muscles These conclusions are strongly supported by the findings of the present study The declining peak resistance response to constrictor fibre activation was as discussed above taken as an indication of an increasing concentration of tissue metabolites Comparing Fig 1 and 2 and Fig 3 and 4 it seems as if an augmented intestinal blood flow is seen when mean nervous resistance response has reached below 80 per cent of control (*i e* between the second and third stimulation periods of Fig 2 and between the first and second ones of Fig 4) Such a decline in nervous responsiveness apparently indicates a large enough accumulation of metabolites to cause an intensified relaxation of the smooth muscles of the intestinal vessels The further decline of nervous responsiveness and additional increase of blood flow between stimulation periods also reflect the accumulation of tissue metabolites throughout the hypotensive periods and to a certain extent also after the hypotension

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The Influence of Contraction Frequency and of a Local Anesthetic (Mepivacaine) on Human Fetal Myocardium

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Abstract

GENNSER G M JOHANSSON and E NILSSON *The influence of contraction frequency and of a local anesthetic (mepivacaine) on human fetal myocardium* Acta physiol scand 1972 *85* 559—568

The relation between contraction frequency and mechanical performance was investigated in isolated perfused heart preparations and in papillary muscles from human fetuses at mid gestation The *influence of mepivacaine on the isometric force and the action potential of the* papillary muscles and on the contractility of the whole hearts was also studied Under the experimental conditions used the isometric force of the papillary muscles increased with frequency within the range 60 to 180 contractions per min The right intraventricular pressure of the isovolumetrically contracting hearts was influenced only to a minor degree by changes in contraction rate between 60 and 150 contractions per min and decreased at higher frequencies Mepivacaine (0 625—10 μg/ml) caused a dose dependent decrease of the active force and a slight reduction of the duration of the action potential in the papillary muscle The effects of contraction frequency and of mepivacaine are discussed in relation to the asynchrony of the ventricular contraction

The isometric contraction force of excised ventricular preparations from mammalian species at 37 C increases with contraction frequency within the range of physiological contraction frequencies *in vivo* (Trautwein and Dudel 1954 a b Koch Weser 1963 Koch Weser and Blinks 1963) However in earlier studies (Andersson Gennser and Nilsson 1970 a b) it has been demonstrated that an increment in contraction rate at frequencies exceeding 100 beats per min exerts a moderate depressant effect on the systolic intraventricular pressure of isolated human fetal hearts and that a similar effect is induced by a local anesthetic mepivacaine in concentrations above 2 5 μg/ml The possibility was discussed that the apparent discrepancy between results reached on excised papillary muscles and on whole hearts at least to some degree might be ascribed to an increasing asynchrony of the contraction of the whole heart at high frequencies The present study was undertaken in order to analyse in further detail the influence of changes in contraction frequency and of mepivacaine on human fetal myocardium The aim of the study was to compare

the effects of the two inotropic interventions on the contractility of papillary muscles where all fibres can be excited within a few milliseconds and on the pressure development in right ventricles where contraction might be significantly affected by asynchronous excitation

Methods

Material mounting of preparations and recording techniques

Papillary muscle Papillary muscles from eight human fetal hearts were obtained at legal abortions The fetal crown heel length varied from 21 to 28 cm The hearts were immediately excised and placed in a modified Ringer s solution of room temperature (composition see below) gassed with 95 % O and 5 % CO A papillary muscle was dissected from the right ventricle together with a piece of the ventricular wall adjacent to the muscle base Only one muscle in each heart was found to have a size (largest diameter 1 0 mm or less) suitable for the present purpose The largest diameter of the muscles averaged 0 6 (0 3—1 0) mm and the mean cross sectional area was 0 29 mm^2 the transverse shape of the muscles being almost circular The dimensions of the muscles were measured in an ocular micrometer at 10× magnification

The preparation was mounted horizontally in a jacketed thermostated bath (volume 1 5 ml) with the ventricle wall connected to a fixed hook and the tendon attached to the elongated peg of a mechano electronic force transducer The compliance of the force transducer was 13×10^{3} $\mu m/dyn$ Intracellular recordings of transmembrane potentials were obtained by means of conventional electrophysiological techniques Micro electrodes (resistances 10—20 MΩ tip potentials less than 5 mV) were used The signals from an intracellular micro-electrode and from the earthed bath were fed into a double sided cathode follower (input capacitance 2 5 pF) and displayed together with the signals from the tension tranducer on a Tectronix 502 A Oscilloscope and recorded by an Elema Schonander Mingograph (flat frequency response to 500 Hz) A Grass Polygraph was used in some experiments The first derivative of the signals from the tension transducer was obtained by an operational amplifier differentiat ing circuit (time constant less than 5 ms which was considered sufficient for the present purpose)

The muscles were stimulated by passing current through an assembly of multiple platinum wires placed at 1 mm intervals along the muscle perpendicular to the long axis of the prepara tion The pulse duration was 2 ms the stimulus intensity was 50 % above the threshold value The resting length of the preparation was chosen slightly below the optimum for active force in the muscle

Whole heart preparation In five experiments whole human fetal hearts were dissected as previously described (Andersson Gennser and Nilsson 1970 a) and perfused *ad modum* Langendorff The dissection was performed in perfusion solution of room temperature equilibrated with 95 % O_2 + 5 % CO the coronary perfusion was established within 10 min after exstirpation of the hearts The crown heel length of the fetus from which these prepara tions were obtained ranged from 17 to 28 cm Care was taken to avoid damaging the myo cardium during the dissection procedure in order not to cause any leakage of the coronary vascular system Perfusion pressure varied between 45 and 50 cm H O in different experiments and was held constant for each heart The pulmonary artery was closed by means of a ligature Right intraventricular pressure during isovolumetric contractions was recorded by a Statham P23AC pressure transducer *via* a polyethylene catheter introduced in the right ventricle through the tricuspidal orifice The compliance of the pressure recording system was 2×10 μl/mm Hg The heart was stimulated through a bipolar platinum electrode placed close to the right atrium To achieve low contraction frequencies the sinus node of the heart was crushed

After mounting the papillary muscle or the heart in the bath an equilibration time of one hour was allowed with the heart contracting spontaneously Recordings were performed alter nately in solution containing mepivacaine and in control solution with an equilibration time of 15 min after each change All recordings refer to steady state conditions at the contraction frequencies studied During each series of recordings at different frequencies the end dia stolic pressure in the right ventricle was constant within ±0 5 mm Hg Within these limits (end diastolic pressure about 2 mm Hg) end diastolic volume was found to vary less than 1 1 % (measured in 3 experiments)

Evaluation of data

Analysis of variance of an unbalanced mixed model (see page 397 in Graybill 1961) was used for statistical evaluation of the electrophysiological data The influence of mepivacaine

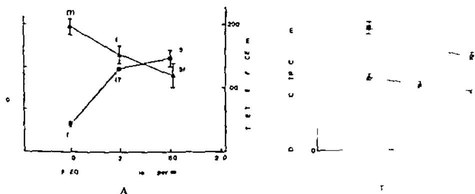


Fig 1 A Active force (●) and time to peak force (▲) of isometric fetal papillary muscles as function of contraction frequency Symbols d r t Figures in brackets indicate number of muscles The active force of each mus l tions per min is taken as unity

Fig 1 B Duration of isometric contraction on 50 % (▲) and 90 % (■) level D n of symbols and figures as in Fig 1 A

and of change of frequency on the papillary muscle contraction was evaluated as follow at each frequency studied the change in active force of a preparation observed during mepivaca e perfusi n was expressed in per cent of the mean value of the active force in the ontrol sol tion before and after perfusion with mepivacaine The confidence limits for the mepivacaine induced changes of force and time course of papillary muscle contraction were calculated for freq encies 60 120 and 180 contractions per min

Solutions

The perfusion solution was of the following composition (mM) NaCl 117 $NaHCO_3$ 20 $MgSO_4$ 1 5 NaH_2PO_4 1 5 KCl 5 $CaCl_2$ 2 and glucose 3 3 Low molecular weight dextran Rheomacrodex® was added to the solution to a final concentration of 2 5 % (w/vol) The $[Ca^{2+}]$ in this solution equals that in 150 mM NaCl solut on containing 0 90 mmoles/l $CaCl_2$ (Andersson Gennser and Nilsson 1970 b) Mepivacaine solutions also contained mepivacaine hydrochloride (Carbocain® mol wt 246) in the following concentrations (μg/ml) 0 625 2 5 and 10 0 All perfusion solutions were equilibrated with 95 % O_2 and 5 % CO_2 in thermostated jacketed glass jars before the inflow into the muscle bath The pH of the solutions (range 7 32—7 41 between different experiments) was kept constant to ±0 02 pH units during each experiment The temperature (37 0 C±0 5 C) was constantly recorded by means of a thermistor in the muscle bath and was not allowed to vary mo e than ±0.3 C during the course of each experiment All chemicals were of analytical grade and glass distilled water was used

Results

The relation between contraction frequency and isometric force of human fetal papillary muscles

Under the experimental conditions used (temperature 37° C concentration of added calcium 2 0 mM) the active force of the papillary muscles increased with increasing contraction frequency within the range 60 to 180 beats per min (see Fig 1 A) In a few preparations the active force was measured at still higher contraction fre quencies Fig 2 shows one such experiment on a thin muscle (largest diameter 0 3 mm) Active force at 240 contractions per min (observe the incomplete dia

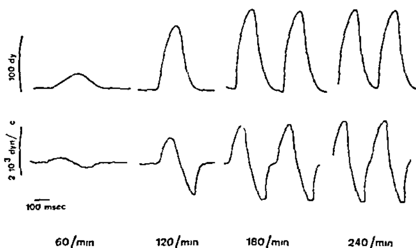


Fig 2 Isometric twitch force (upper trace) and dp/dt (lower trace) of a human fetal papillary muscle from the right ventricle at different contraction frequencies Muscle length 3 2 mm, largest diameter 0 4 mm. Resting force 58 dyn Note that at 240 contractions per min relaxation is incomplete and that $(dp/dt)_{max}$ is greater at 240 than at 180 contractions per min

relaxation) is equal to that at 180 contractions per min and the maximum rate of force development $(dp/dt)_m$ is slightly greater at the higher frequency —Fig 1 A also shows the time from onset of contraction to peak twitch force Fig 1 B hows the duration of the contraction cycle at 50 % and 90 % level As can be seen with increasing frequency peak isometric force is attained at an earlier time after the onset of contraction and the duration of the contraction cycle is abbreviated.

The frequency induced changes of active force had a slow time course The times necessary for reaching half of the force change caused by alterations in frequency from 60 to 120 and from 120 to 60 contractions per min were 30 ± 6 (n = 6) and 27 ± 7 (n = 4) (mean ± S E) respectively

The relation between contraction frequency and right intraventricular pressure of human fetal hearts

The frequency-dependence of active systolic pressure within the right ventricle of fetal hearts was strikingly different from that of active force of excised papillary muscles Fig 3 A illustrates recordings of right intraventricular pressure of one heart The pressure was recorded during isovolumetric contractions *via* a catheter inserted through the tricuspidal orifice (see Methods) It should be observed (see Methods) that end diastolic volume was constant to ±0 5 mm Hg during each series of contraction frequencies which seems to exclude that the results obtained were significantly affected by changes in ventricular volume The intraventricular systolic pressure was only slightly affected by changes in contraction rate within the range 60

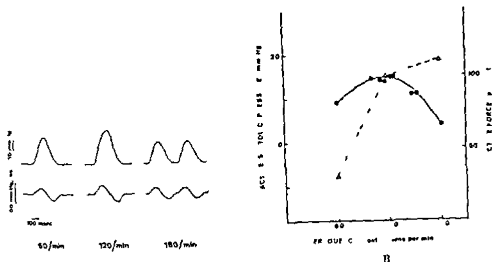


Fig 3 A Recordings of right intraventricular pressure (upper trace) and its rate of change (lower trace) of a fetal heart preparation at 3 contraction frequencies End diastolic pressure 1 mm Hg at all contractions

Fig 3 B Active systolic pressure (●——●) in right ventricle at different contraction frequencies Same preparation as in Fig 3 A For comparison is shown the force frequency relation (Δ Δ) of isolated fetal papillary muscles from the right ventricle (same data as given in Fig 1 A)

to 150 beats per min (Fig 3 B) At higher frequencies a marked fall in active systolic pressure occurred In Fig 3 B is given for comparison both the intraventricular pressure of a right ventricle and the isometric force of isolated papillary muscles as a function of contraction frequency The discrepancy between the mechanical performance of the 2 types of preparations is apparent Above 120 contraction per min the active systolic pressure of the ventricle decreased with frequency whereas the active force of isolated papillary muscles increased

Results similar to those presented in Fig 3 were obtained by pressure recordings in 4 other experiments

Influence of mepivacaine on whole hearts and papillary muscles

Mepivacaine in concentration 0 625—10 0 μg/ml reduced the active force of papillary muscles Fig 4 reports the relation between force reduction contraction frequency and mepivacaine concentration in the perfusion fluid At each particular frequency the decrease in active force is expressed in per cent of the force produced by the muscles at the same frequency in the control solution The effect induced by mepivacaine was found to be dependent on its concentration in the solution The 3 concentrations of mepivacaine tested differ by a factor of 4 As can bee seen there is only a slight difference between the effects of 0 625 and 2 5 μg/ml whereas the effect induced by 10 μg/ml far exceeds that of 2 5 μg/ml The force reduction induced by mepivacaine in all three concentrations tended to be less pronounced

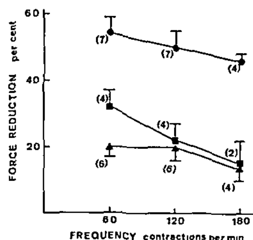


Fig 4 Effects of mepivacaine in concentrations 0 625 μg/ml (▲) 2 5 μg/ml (■) and 10 0 μg/ml (●) on active force of isometrically contracting papillary muscles from fetal right ventricle at different contraction frequencies The force reduction in mepivacaine solution at each frequency is expressed in per cent of the force at the same frequency in control solution Symbols denote mean±S E figures in brackets number of muscles The force reduction between the three frequencies tested are not significantly different in any of the 3 mepivacaine solutions

TABLE I Time course of contraction of papillary muscles Mean±S E Figures in brackets denote number of muscles

	Time to peak tension	Duration of contraction			
		50 level		90 , level	
	120/min	60/min	120/min	60/min	1?0/min
Control	*15? 7±10 7* (7)	*165 1±7 5* (8)	*146 2±4 5* (8)	*277 8±14 8* (8)	*235 6±7 7* (8)
Mepivacaine 10 μg/ml	134 0± 6 7 (7)	155 1±5 5 (7)	140 1±6 1 (8)	262 6±10 4 (7)	2?6 5±8 3 (8)
Significance of difference	significant at 2 5 level	significant at 5 % level	significant at 2 5 % level	not significant at 10 level	significant at 5 % level

TABLE II Electrophysiological parameters of papillary muscles at 60 contractions per min Mepivacaine concentration 10 μg/ml Mean±S E Data based on 3—7 impalements in each case

Experiment no	Action potential duration (ms)				resting potential (mV)	
	50 level		90 level			
	Control	Mepivacaine	Control	Mepivacaine	Control	Mepivacaine
1	220 6±1 4	201 0±0 6	30(7±1 7	279 5±0 5	73 5±3 2	72 7±2 7
2	237 0±8 6	211 2 ±3 8	325 0±12 9	29? 0±3 5	72 7±0 3	71 0±1 0
3	210 0±2 2	187 5±3 2	294 5±5 7	277 6±2 3	72 8±2 7	68 4±2 5
4	2?3 3±5 5	168 8±17 6	298 8±4 3	253 8±15 5	75 5±4 5	76 5±3 5
Significance of difference	significant at 2 5 % level		significant at 5 level		not significant at 20 level	

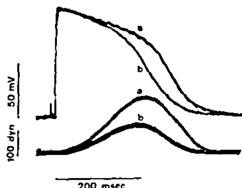


Fig 5 Photographically superimposed traces of action potential (upper records) and isometric force (lower records) of an excised human fetal papillary muscle (length 4 6 mm largest diameter 0 6 mm) before (a) and after (b) addition of mepivacaine 10 μg/ml to the perfusion solution Frequency 60 contractions per min

at high contraction frequencies The observed difference between the effects at the three frequencies is however not statistically significant Table I shows that in the papillary muscles at a frequency of 120 contractions per min mepivacaine in concentration 10 μg/ml caused a slight reduction of the time from onset of contraction to peak isometric force and a minor decrease of the duration of the contraction cycle

Table II summarizes results of action potential recordings from papillary muscles in ordinary perfusion solution and in perfusion solution containing mepivacaine 10 μg/ml Mepivacaine in this concentration induced a slight reduction of action potential duration but no significant change of resting membrane potential Fig 5 shows typical oscilloscope traces from one experiment The reduction of active force and the shortening of the time to peak force as well as the abbreviation of the action potential duration during mepivacaine perfusion is apparent

The effect of mepivacaine on the intraventricular pressure in the right ventricle at constant frequency was investigated in four whole heart preparations In accordance with earlier observations (Andersson Gennser and Nilsson 1970 b) mepivacaine in concentration 10 μg/ml caused a marked reduction of intraventricular pressure This mepivacaine induced effect defined as reduction of pressure in per cent of pressure in the control solution at the particular frequency increased with increasing frequency Mepivacaine tended to cause irreversible effects on the mechanical output of the hearts when stimulated at frequencies exceeding the spontaneous rate At any given contraction frequency no consistent change in the duration of the contractile cycle at the 50 % and 90 % level was observed after addition of mepivacaine 10 μg/ml to the perfusion solution

Discussion

In a previous study it was shown that at 30° C the active force of human fetal papillary muscles increased with contraction frequency up to 84 contractions per min (Gennser and Nilsson 1968) The frequency optimum for active force is known to increase with temperature (Trautwein and Dudel 1954 b) and thus agrees

with the results of the present study, in which force increased with frequency up to 180 contractions per min The present study also indicates that the frequency dependence of isolated papillary muscles differ from that of whole hearts Within the range 60 to 150 beats per min, maximum isovolumetric pressure in the *right* ventricle was only slightly affected by alterations in frequency, whereas a fall in active pressure occurred at higher frequencies These results are in fair agreement with pressure recordings from the *left* ventricle of isolated human fetal hearts (Andersson Gennser and Nilsson 1970 a) It should be noted that the same perfusion solution was used in studies both on intact fetal ventricles and on papillary muscles This seems to preclude the possibility that biologically active substances reaching the heart *via* the circulation can be responsible for the observed difference in frequency dependence between the mechanical output of the two types of preparations The fact that the mechanical output increased with frequency in papillary muscles but not in intact ventricles is probably not attributable to difference in modes of perfusion of the 2 types of preparations If anything it is resonable to believe that the coronary perfusion of the intact ventricle would provide a more effective oxygenation of the myocardium than mere surface perfusion of the papillary muscle

As previously discussed (Andersson Gennser and Nilsson 1970 a Johannsson and ilsson 1972) the *asynchronous* contraction of an intact ventricle provides an ex tion for the different frequency dependence of the mechanical performance of ict ventricles and of excised ventricular preparations such as papillary muscles ie total excitation time of the fetal left ventricle amounts to approximately 10 ms (Durrer *et al* 1960) and would be expected to remain constant within the frequency range analysed in the present study or to increase slightly at high frequencies due to decreased intraventricular conduction velocity (Gennser and Nilsson 1970) With increasing frequency the rate of rise of the mechanical activity in heart muscle fibres is enhanced (Gennser and Nilsson 1968 Edman and Nilsson 1969) and the duration of the mechanical activity shortened (Fig 1 B) Therefore the degree of asynchrony of the ventricular contraction might be expected to increase with increasing frequency The increased asynchrony of the ventricular contraction would tend to shift the frequency optimum for intraventricular pressure to a lower value than that optimum for isometric force of excised preparations (papillary muscles) Furthermore the asynchronous contraction would prolong the duration of the mechanical activity of the ventricle compared to that of the heart cells The net result will be that the asyn hronous contraction of the ventricle moderates the *cellular effects of an increased contraction rate i e enhanced contractility* and decreased duration of the contraction cycle (*cf* discussion in Johannsson and Nil on 1972)

The results of the present study demonstrate that mepivacaine in concentrations that can be reached in fetal blood during paracervical blockade associated with signs of cardiovascular distress (Gordon 1968 Martin *et al* 1969 Asling *et al* 1970 Teramo and Rajamaki 1971) depresses contractility in papillary muscles of human fetal hearts The mepivacaine induced reduction of force of papillary muscles (ex

pressed in per cent of the active force in the control solution at the respective frequency) did not change significantly with contraction rate On the other hand in the intact isolated fetal heart the negative effect of mepivacaine on the mechanical performance of the ventricle (measured as per cent decrease in intraventricular pressure) increased with frequency (Fig 4 in Andersson Gennser and Nilsson 1970 c and confirmed in the present report) The greater effect of mepivacaine exhibited at high than at low contraction frequencies in whole ventricles is possibly attributable to the fact that mepivacaine reduces intraventricular conduction velocity (Andersson Gennser and Nilsson 1970 c) This would enhance the degree of asynchrony of the ventricular contraction and consequently diminish the mechanical performance of the ventricle more so at high than at low contraction frequencies (*cf* above)

A discussion of the mechanisms of the mepivacaine effect is beyond the scope of this report It is unlikely however that the small reduction of the action potential duration caused by mepivacaine in the present study (10—14 % of control at mepivacaine 10 μg/ml and 60 contractions per min) is the only explanation for the observed depressant effect on contractile force (53 % of control at the same mepivacaine concentration and contraction frequency) These results seem to implicate an action exerted by mepivacaine on the excitation-contraction coupling in the myocardial cells beyond the effect on the action potential *per se*

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From the Psychological Laboratories University of Stockholm Sweden

Sex Differences in the Catecholamine Output of Children

By

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Abstract

JOHANSSON G *Sex differences in the catecholamine output of children* Acta physiol scand 1972 85 569—572

Urinary excretion of catecholamines was studied in 240 normal healthy children 12 years of age during a passive condition (film exposure) and an active condition (arithmetic test) at school No sex differences in catecholamine excretion were found during the passive condition whereas during the work period boys excreted significantly more adrenaline and noradrenaline than girls A comparison is made between catecholamine excretion levels of the children and adult subjects examined in other studies

Circulating catecholamines in particular adrenaline have been shown to play an important part in the regulation of behaviour of normal healthy human subjects (see reviews by Euler 1967 and Frankenhaeuser 1971) Most investigations have been performed on adult male subjects and relations between catecholamine output sex and age in humans have been incompletely surveyed Some data suggest that women excrete less adrenaline and noradrenaline than men (Karki 1956 Lambert *et al* 1969) For an adequate comparison between sexes it is however necessary to take into account differences in body weight This was done by Karki (1956) who studied urinary excretion of adrenaline and noradrenaline in five different age groups of men and women Urine samples were obtained for a 24 h period during which the subjects activities were *not regulated* or controlled Only non significant sex differences remained after correction for body weight

The present report is concerned with differences in catecholamine excretion between boys and girls in two different carefully controlled conditions The study was performed as part of a longitudinal study of adjustment behaviour and performance in school children (Magnusson Duner and Beckne 1965) Relations between catecholamine measures and behavioural data have been reported separately (Johansson Frankenhaeuser and Magnusson 1971)

Methods

9 classes of grade 6 (age of pupils about 12 years) were sampled from a total of 39 classes The present sample comprised 252 pupils of which 240 were present at school during the days of investigation The final data analysis contains data for 177 pupils For the remaining 63 pupils no or only one excretion value was obtained for the following reasons the pupils inability to void on one or more occasions (53 cases) menstruation period (6 cases) or technical mishaps during the analysis of urine samples (4 cases)

The 9 classes of children were studied on separate days For each class the investigation took place in the ordinary classroom between 8 15 and 10 50 a. m First each child emptied his bladder at a near by toilet and noted the time Then all children gathered in the class room where they viewed an emotionally neutral motion picture for about 42 min The film session will be called the passive condition After a pause for urine sampling the active condition followed in which the children performed an attention demanding mental arithmetic test for 42 min Thereafter urine samples were again collected To secure prompt micturation each subject drank 100 ml of water before each session

Immediately after urine collection the volume of each urine sample was measured and pH adjusted to about 3 by addition of 2 N HCl Samples were frozen and stored at —18 C until analyzed by the fluorimetric technique of Euler and Lishajko (1961)

The body weight of each child was measured within a few weeks either before or after the collection of urine samples

Results

For 27 of the 63 children from whom urine samples were not obtained at both occasions excretion values were obtained for the two periods together ponding values were calculated for those pupils who gave complete sets of ples This two period measure made it possible to compare the catecholamine cretion of the one sample group with that of the two sample group A two tailed t test showed that there was no significant difference between the mean total excretion in the 2 groups This was true for both sexes and for both catecholamines It was concluded that the loss of data was random rather than systematic

Fig 1 shows the mean adrenaline and noradrenaline excretion of boys and girls respectively during each of the two conditions Excretion values are expressed in ng/min/kg b w It is seen that the girls excreted less adrenaline than the boys (left diagram) During the active period the difference was statistically significant ($t = 3\ 6$ $p < 0\ 001$) The excretion rate of noradrenaline (right diagram) was also slightly lower for girls than for boys during both conditions and again the difference is significant only for the active period ($t = 2\ 5$ $p < 0\ 05$)

The magnitude of change was investigated by calculation of the difference in catecholamine output between active and passive condition A test of significance showed that the boys increased their adrenaline excretion significantly more than the girls ($t = 2\ 196$ $p < 0\ 05$)

The magnitude of noradrenaline change did not differ between the sexes

Discussion

On the basis of earlier comparisons of catecholamine excretion of men and women it has been suggested that when correction for body weight is made no differences exist between the sexes in either adrenaline or noradrenaline excretion The results

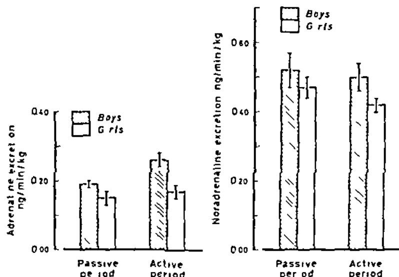


Fig 1 Mean excretion rate of adrenaline and noradrenaline by boys (n = 99) and girls (n = 78) in a passive situation (film exposure) and an active situation (test of arithmetic) Values corrected for body weight

of the present study indicate that at least among 12 year old children there is a difference between sexes which is manifested only under certain conditions Thus during the passive condition of watching a motion picture the excretion levels of boys and girls did not differ significantly during a test of arithmetic involving a moderate mental work load the boys secreted significantly more adrenaline and noradrenaline than the girls Corresponding data for adults do not yet seem to be available and hence it is not known at present whether the sex difference in catecholamine output occurs only at certain age levels

Boys and girls differed only slightly in the psychological variables and background variables investigated and it appears unlikely that the sex differences in catecholamine secretion may be accounted for in terms of psychological differences only However the girls were superior to the boys in accuracy of performance during the active period (Johansson 1970) and they also had significantly lower scores than the boys in a factor scale measuring *apprehension for school work* Such differences might have influenced the way in which boys and girls appraised the test situation and indirectly their arousal level (*cf* Lazarus 1969) In view of the fact that the stress involved may be considered rather mild the children having been informed that their performance in the test would in no way influence their school record a possible difference in appraisal of threat is not likely to be the only factor responsible for the difference in catecholamine secretion between the sexes

A comparison between the present data and previous data on adult subject gests that children secrete more adrenaline than adults According to cal

made by the present author on data available from previous studies (Frankenhaeuser and Rissler 1970 Lambert *et al* 1969, Frankenhaeuser *et al* 1971 a b Nordheden 1971) the mean excretion rate of adrenaline among adults during various passive conditions has generally not exceeded 0 10 ng/min/kg b w Both boys and girls in the present study had higher levels of excretion As to the excretion of noradrenaline there was no clear difference between the children in this study and adult subjects in previous studies

The data reported in this paper were collected within the frame of a long term study of school children led by Professor D Magnusson University of Stockholm and financially supported by the National Swedish Board of Education The work was facilitated by a grant from the University of Stockholm and a grant to Professor M Frankenhaeuser from the Swedish Medical Research Council (No 40X—997)

B Nordheden B A assisted in the data collection and the catecholamine analyses were performed by Miss B Linell

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Acta physiol scand 1972 85 573—576

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Increased Renal Excretion of Noradrenaline in Rats after Treatment with Prostaglandin Synthesis Inhibitor Indomethacin

By

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Abstract

JUNSTAD M and Å WENNMALM *Increased renal excretion of noradrenaline in rats after treatment with prostaglandin synthesis inhibitor indomethacin* Acta physiol scand 1972 *85* 573—576

The renal excretion of noradrenaline (NA) during prolonged treatment with the prostaglandin synthesis inhibitor indomethacin (Indomee®) was studied in the rat. The excretion of NA was increased by about 50 % during treatment with 0 5—2 0 mg/kg b w of indomethacin with a slight dose dependence The results support the hypothesis based on studies in isolated perfused organs that the *in vivo* release of NA from adrenergic nerve endings is limited by an endogenous prostaglandin mediated braking system

Prostaglandins of the E series have been shown to inhibit the release of NA from adrenergic nerve endings in various tissues and species (*cf* Hedqvist 1970, Wennmalm 1971) Since prostaglandins are widely distributed in animal tissues (Bergstrom Carlson and Weeks 1968) and since they are released in response to sympathetic nerve stimulation (Davies Horton and Withrington 1968 Gilmore Vane and Wyllie 1968) it has been proposed (Hedqvist 1969) that endogenous prostaglandins modulate the normal release of NA from adrenergic neurons Such a modulating action by endogenous prostaglandins has been shown in several isolated, sympathetically innervated preparations (for references see discussion) Since these studies were performed on isolated organs the logical extension was to investigate if the same action by endogenous prostaglandin could be demonstrated in intact animals

The antiflogistic drug indomethacin has recently been shown to inhibit the synthesis of prostaglandins (Vane 1971) Since the renal excretion of NA appears to reflect the release of transmitter from the adrenergic nerve endings (Leduc 1961) it was of interest to study if administration of indomethacin to intact animals is accompanied by an increased renal excretion of NA

made by the present author on data available from previous studies (Frankenhaeuser and Rissler 1970 Lambert *et al* 1969 Frankenhaeuser *et al* 1971 a b Nordheden 1971) the mean excretion rate of adrenaline among adults during various passive conditions has generally not exceeded 0 10 ng/min/kg b w Both boys and girls in the present study had higher levels of excretion As to the excretion of noradrenaline there was no clear difference between the children in this study and adult subjects in previous studies

The data reported in this paper were collected within the frame of a long term study of school children led by Professor D Magnusson University of Stockholm and financially supported by the National Swedish Board of Education The work was facilitated by a grant from the University of Stockholm and a grant to Professor M Frankenhaeuser from the Swedish Medical Research Council (No 40X—997)
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intact animals, suggests that the prostaglandin brake on the release of NA from sympathetic nerve endings operates also *in vivo*

In parallel with this study, Stjarne (1971) has investigated the effect of orally administered indomethacin on the renal excretion of catecholamines No increase in urinary catecholamines was found in this study, as long as the rats were kept at room temperature It seems possible that the discrepancy might be explained in terms of the different techniques of drug administration However, Stjarne found that on exposure to intermittent cold, the normal increase in urinary NA (*cf* Leduc 1961) was further augmented on oral administration of indomethacin The relative increase in urinary NA was of the same magnitude as that reported in this paper

In conclusion the results reported here indicate that endogenous prostaglandins exert a braking action on the process of release of NA from sympathetic nerve endings not only in isolated preparations but also in the intact animal The present study shows that evidence for such an *in vivo* mechanism can be obtained even if the renal excretion of NA immediately before indomethacin treatment is only moderately increased by stress

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